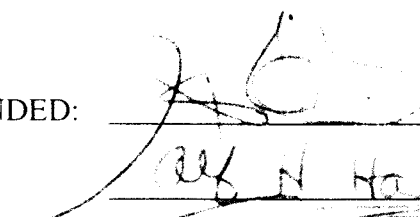


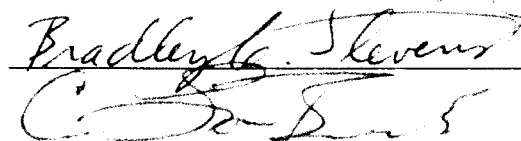
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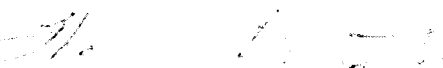
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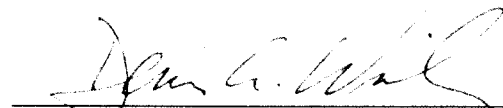
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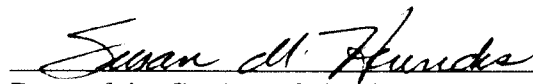

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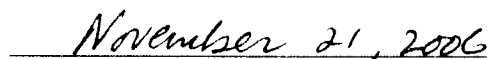

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PHYSIOLOGICAL AND BEHAVIORAL RESPONSES OF TANNER CRABS
(*CHIONOECETES BAIRDI*) TO HANDLING, EMERSION AND TEMPERATURE

A
THESIS

Presented to the Faculty
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By
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ABSTRACT

Commercially harvested Tanner crabs (*Chionoecetes bairdi*) are exposed to physical stressors during capture and sorting including changes in temperature and oxygen availability. This study characterizes the physiological and behavioral responses of Tanner crabs exposed to air (emersion) at 8 and -15°C for various durations. Concentrations of glucose and lactate in hemolymph measured between 30 and 120 min following emersion for 45 min differed between animals exposed to 8 or -15°C. Glucose concentrations were higher among animals emersed at 8°C than those exposed to -15°C within the intervals sampled. Lactate concentrations were unchanged at intervals following emersion at 8°C, while they were elevated at 120 min following emersion at -15°C. Rates of oxygen consumption (VO_2) increased immediately following 15, 30, and 45 min emersion at 8°C, whereas 30 and 45 min emersion at -15°C resulted in depressed VO_2 . All crabs survived handling and emersion at 8°C, while exposure to -15°C resulted in increased mortality. Thus, differences among physiological parameters corresponded with differences in percentage survival between the two temperature treatments. While not providing a causal relationship between survival and physiology, the metabolic responses of Tanner crabs following a simulated capture protocol provide a predictive index of subsequent survival.

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CHAPTER 1 : GENERAL INTRODUCTION

TANNER CRAB LIFE HISTORY

Tanner crabs (*Chionoecetes bairdi*; Rathbun, 1924) are abundant in the North Pacific Ocean from the coast of Oregon to the Gulf of Alaska and in the Bering Sea where they inhabit depths ranging from the subtidal to > 400 meters (Jadamec et al., 1999). In the summer of 1991, maximum densities of Tanner crabs were observed around 140-150 meters in a southeast Alaska fjord (Zhou and Shirley, 1998). Generally, juvenile Tanner crabs are found in shallow inshore waters and, as they molt into adulthood, they migrate to deeper waters (Stevens et al., 1993b). Because they are exposed to a wide range of depths throughout their lifecycle, they are also exposed to a relatively wide temperature range. However, the preferred water temperatures and upper thermal limits of Tanner crabs have not currently been determined. Tanner crabs captured at a depth of 75 meters were dwelling in water temperatures between 3°C in the winter and 10°C in the summer-fall season (Paul and Paul, 2001a).

Tanner crabs mate once per year beginning in winter and ending in spring. Pubescent females are the first to mate (January-May). They generally remain in shallower waters (< 30m) and form isolated mating pairs with the smaller males (Stevens et al., 1993b). Multiparous females mate later in the year (April-June) and, as they gather in deeper waters (over 150 meters on the continental shelf), they mate in aggregate with the larger males (Stevens et al., 1994). Mating aggregations consist of hundreds of reproductive females mounded up to a meter high and several meters long and males scattered on the seafloor surrounding the mounds.

When a male detects a receptive female, he grasps her by the legs and she faces him in a pre-mating embrace (Adams, 1982; Donaldson and Adams, 1989). A male mating with a pubescent female assists the female throughout her molt to maturity and

transfers spermatophores into the female's gonopores when she is in a soft-shell state. However, if the female is multiparous and has undergone her terminal molt, the male internally fertilizes the female in her hard-shell state. The male continues to grasp the female until the fertilized eggs are extruded under the abdominal flap to ensure successful mating (Jadamec et al., 1999). Females can store sperm in their spermathecae and produce viable eggs with 1 or 2 year-old sperm, thus enabling production of multiple broods without proximal mating (Adams and Paul, 1983; Paul, 1984). However, only one clutch is produced per year and eggs are brooded for about one year until they hatch as larvae prior to the next mating (Paul, 1984; Paul and Adams, 1984).

Several hundred thousand Tanner crab larvae (0.5 to 1 mm in carapace length) are released from each gravid female between late winter and early summer and begin development as plankton (Jadamec et al., 1999). The planktonic zoea are distributed in the upper 20 meters of the water column where they remain for 2 months (Incze et al., 1987). The megalop or settling stage (6-7 mm in carapace length) migrates to the sea floor between 1 and 10 months of age and molts into the first instar (3.5 mm in carapace width), which resembles a small adult (Jadamec et al., 1999).

Small crabs probably settle in shallow inshore waters and migrate to deeper waters with age. Juvenile Tanner crabs have been noted to molt in mass at depths < 20 meters in southeast Alaska during the spring molting season (Stone, 1999). The authors suggested that by migrating to shallow water, Tanner crabs avoided deepwater predators such as groundfish and other crab species, including hard-shelled Tanner crabs, while vulnerable in their soft shell form. After molting to maturity and mating at least once, adult males and females move to deeper waters (> 100 m) where they remain the rest of their life (Stevens et al., 1993b).

Molting, or ecdysis, for crustaceans requires shedding of the hard shell exoskeleton in order to expand body mass. Between 3 and 6 weeks prior to ecdysis, the

shell decalcifies. During the molting process, the cephalothorax is elevated causing the ecdysial suture to break, thus allowing the soft shell animal to exit the old exoskeleton (Jadamec et al., 1999). Soft shell crabs are defenseless and barely capable of moving, but as the shell hardens, the crab recovers all its abilities (Paul et al., 1995). Calcification of the shell is a fairly slow process starting within a few hours post-ecdysis and lasting between 16 and 71 days (Adams, 1982).

Growth is defined as the increase in size gained per molt and the frequency of molts (Donaldson et al., 1981). The rate of growth on a percentage basis decreases with increased size of crabs regardless of sex; that is, small crabs grow relatively more in size at a molt than larger crabs (Paul and Paul, 2001b). The frequency of molt is both size and maturity dependent. The intermolt period, time between molts, for male Tanner crabs is shorter for smaller crabs than larger crabs, indicating that small crabs molt more frequently. Also, the intermolt period is shorter for juvenile crabs (not bearing spermatophores) than adolescent crabs (bearing spermatophores) of similar size, indicating that juvenile crabs molt more frequently (Paul and Paul, 2001b).

Adult females are generally smaller than adult males. In Kodiak waters, the average carapace width (CW) for adult female Tanner crabs is 94 mm compared to 150 mm for adult male Tanner crabs (Donaldson et al., 1981). During the Bering Sea Tanner crab fishery, the largest females measured 122 mm in CW, while the largest males measured 182 mm in CW between 1991 and 1994 (Tracy, 1995).

Females grow for about 5 years and undergo their final molt at the 13th instar as they attain maturity. Males attain functional maturity after a period of 6 years and at the 18th instar (Jadamec et al., 1999). However, it is still debated whether male Tanner crabs undergo a terminal molt, like females do, after they have reached functional maturity or if they continue to grow (Conan and Comeau, 1986; Donaldson and Johnson, 1988). Nonetheless, the functional maturity of a male Tanner crab can be determined by its

enlarged claws. As males molt to maturity they grow allometrically such that carapace width (CW) and height of chela (CH) increase disproportionately (Stevens et al., 1993a). Larger claws are thought to provide advantages in grasping and handling females in a mating embrace. Juvenile male Tanner crabs generally do not participate in mating and have a small CH relative to CW, while the majority of males that exhibit grasping behaviors have large claws. Therefore, morphometric maturity is likely a prerequisite for functional maturity in male Tanner crabs. Functionally mature male crabs have a relatively large chela to body size ratio such that $CH/CW > 0.17$ (Stevens et al., 1993a). In the present study, mature male Tanner crabs were selected using the same ratio number.

TANNER CRAB FISHERIES BACKGROUND

The commercial harvest of Tanner crabs in Alaskan waters is a significant industry. The greatest catch of Tanner crabs was in 1977 in the Bering Sea when 78.3 million pounds of crab were landed. To put this in perspective, in the same year in Bristol Bay, 11.9 million pounds of snow crab (*Chionoecetes opilio*) and 70 million pounds of red king crab (*Paralithodes camtschaticus*) were landed (Ruccio, 2003). Economically, the red king crab industry yields the greatest ex-vessel price per pound. In 2002, prices reached \$6.27/lb for Aleutian Islands red king crabs compared to \$2.21/lb for Kodiak Tanner crabs and \$1.40/lb for Bering Sea snow crabs (ADFG, 2004). Even though the Tanner crab fishery developed later than the red king crab fishery it quickly grew and contributed millions of pounds of crab for many consecutive years. However, stocks of Tanner crabs have undergone extreme fluctuations in recent years and are currently so low that commercial fisheries have been closed in the Bering Sea and Aleutians since the mid-1990s and are only sporadically open in some areas in the Gulf of Alaska.

Fisheries regulations are implemented to manage stocks of Tanner crabs to ensure long-term sustainability of the species. Pot and trawl surveys are conducted by the Alaska

Department of Fish and Game (ADFG) to estimate abundance of mature males, predict recruitment, and determine levels of annual harvest (Cavin et al., 2002). A season closure was established in 1975 as April 30 to protect crabs at the onset of mating and molting in the late springtime, and in 1976 a minimum CW of 140 mm (5.5 inches) was established to allow crabs to mate at least once in their life before becoming legally harvestable by the fishery (Cavin et al., 2002).

Commercial fishing for Tanner crab may occur in Southeastern Alaska, Yakutat, Kodiak, and South Peninsula areas on January 15 once harvest strategies are met for each district (Iverson, 2001). In the Kodiak district, the Alaskan Board of Fisheries (BOF) established harvest strategies in 1999 such that a threshold abundance of mature male crabs must be met or exceeded in order for fisheries to occur and guideline harvest levels (GHL) must be greater than 400,000 pounds of crab for the whole district (Ruccio, 2003). GHL are based upon abundance estimates and differ between districts and years.

The Tanner crab fisheries began in 1967 in the Kodiak District. This district encompasses waters around the Kodiak Archipelago from 1 to 200 nm off shore and is divided into eight management sections - Northeast, Eastside, Southeast, Southwest, Westside, North Mainland, South Mainland, and Semidi Islands (Figure 1.1). Tanner crab fisheries can be open in some sections and closed in others depending on estimates of population size and pre-season GHL per section (Iverson, 2001; Ruccio, 2003). Catches, in the Kodiak District, peaked in 1978 with over 33 million pounds of crab landed and averaged 20.9 million pounds annually between 1970 and 1980. However, over the next 15 years, numbers of Tanner crab rapidly declined with harvests totaling only 1.2 million pounds in 1994. The Kodiak Tanner crab fishery closed in 1995 and remained so for the next 6 years. In 2001, it was reopened but population levels of Tanner crab remain relatively depressed. The 2003 harvest of Tanner crabs in the Kodiak district totaled 510,000 pounds of crab for an ex-vessel value of \$1.18 million (Cavin et al., 2002; ADFG, 2004; Ruccio, 2003).

Boats may use pots or traps of limited dimensions (10'x10'x 42'') to legally exploit Tanner crabs. Neither trawling nor ring nets are legal types of gear in the Kodiak District. An escape panel of at least 7 ¼ inches stretched mesh or a minimum of 4 escape rings of at least 5 inches in diameter are required to allow undersized crabs to exit. The maximum number of pots allowed per vessel can increase as the GHL increases. Pots are baited and dropped to the sea bottom and left for a limited duration after which pots are retrieved and crabs are sorted on deck by species, sex, and size. Only large adult male crabs are legally harvested. Sub-legal male (≤ 140 mm CW) and female Tanner crabs, as well as all other species, are considered bycatch and are returned to the sea (Iverson, 2001; Ruccio, 2003).

The number of Tanner crabs caught and returned to the sea is substantial. During the 1994 Bering Sea Tanner crab fishery, an estimated 3.8 million legal Tanner crabs were retained while 5.9 million sub-legal males and 3.6 million female Tanner crabs were caught and released (Tracy, 1995). Other fisheries yield considerable numbers of Tanner crab bycatch such as the Bering Sea scallop fisheries and snow crab fisheries (Rosenkranz, 2002; Tracy, 1995). During the 1994 Bering Sea snow crab fishery, sub-legal male Tanner crabs constituted the largest portion of crab bycatch and were estimated at 6.4 million followed by 2.3 million females and 0.4 million legal males (Tracy, 1995). Given these numbers, any decrease in viability or fitness of animals captured and subsequently returned to the sea may have measurable impacts on future recruitment. Hence, changes in the methods of harvest that increase survivorship of bycatch have the potential for tremendous benefit to sustainable fisheries.

The Tanner crab fishery occurs in the winter months and therefore animals may be exposed to extreme weather and sea conditions as they are brought up on deck. The typical conditions in the Bering Sea between January and March include average monthly air temperatures ranging from -0.6 to -3.6°C and average monthly wind speeds ranging from 9 to 11 m s⁻¹ (Warrenchuk and Shirley, 2002a). During the sorting process, crabs are

exposed to air for a few minutes to hours depending on the type of fishery and the number of crabs caught. Animals harvested in pots are handled relatively quickly, whereas those caught as bycatch during trawling operations can remain on deck for hours (Stevens, 1990). Rough handling on board and contact with heavy gear may cause severe injuries. Investigating the effects of any of these stressors on survival of Tanner crabs returned to the sea can be used to improve estimates of bycatch mortality, a parameter of importance to fisheries managers.

Handling, air exposure and low temperatures either alone or in combination have adverse effects on the health, performance, and viability of crabs. Tanner crabs exposed to air and low temperatures had reduced righting response, increased limb autotomy (leg dropped in response to limb injury or other stress), and increased mortality (Carls and O'Clair, 1995). Similarly, the effects of windchill combined with subfreezing temperature on the congeneric snow crabs induced high rates of limb loss, reduced righting response, and resulted in 40-100% mortality following severe treatments (Warrenchuk and Shirley, 2002b). MacIntosh et al. (1996) reported that 85% of Tanner crabs experimentally injured in the legs had autotomized their limbs within 24 hours. Injuries were meant to simulate handling conditions on fishing boats and included pinched, bent, and cracked limbs. Such injuries, even if not immediately fatal, may render an individual more vulnerable to predation or less likely to reproduce. During a commercial sole trawl, only 22% of all Tanner crab bycatch ($n = 16,498$) survived after 48 hours observation (Stevens, 1990). While these data demonstrate a measurable impact of environmental stressors on the vitality of Tanner crabs, little data describing their physiological responses to various stressors is available.

Understanding metabolism of crabs following various stressors can provide a quantitative measure of the response of crabs to more subtle stressors. These stressors do not necessarily kill crabs, but they may cause animals to incur a metabolic cost or impair their ability to escape a subsequent stressor. Quantifying physiological responses to environmental and physical insults might be helpful in predicting performance and

vitality of crabs caught and released. Moreover, this information could prove useful in predicting health and subsequent survivorship of individual Tanner crabs selected for live delivery to markets.

TANNER CRAB BIOLOGY

Metabolic physiology

Metabolism integrates all chemical processes and energy transformation that sustain a living organism, and can be summarized by two main mechanisms - energy extraction and energy use (Schmidt-Nielson, 1997). Animals extract energy by breaking down food components such as carbohydrates, amino acids, and fatty acids through processes known as catabolism. This energy is stored in the form of adenosine triphosphate (ATP) and cells use it to biosynthesize new macromolecules needed by the organism through processes known as anabolism. ATP is also used for other physiological activities such as active transport and movement while some energy is lost in the form of heat. It appears that in crustaceans, the principal biochemical pathways that produce ATP are similar to vertebrates (Chang and O'Connor, 1983).

Glucose is found to be the major component of circulating carbohydrates in crustacean hemolymph and is a major source of potential energy (Chang and O'Connor, 1983; Schmidt-Nielson, 1997). In the cytosol of the cell, glucose undergoes glycolysis to form pyruvate. This pathway yields 2 molecules of ATP and does not require oxygen. Pyruvate can either be used in oxidative respiration or be reduced to lactate depending on the levels of oxygen present. In the mitochondrial matrix where oxidative metabolism takes place, the acetyl group of pyruvate combines with coenzyme A to form acetyl coenzyme A (acetylCoA), which is then fed into the citric acid cycle. High-energy electrons that are released during this process are fed into the electron transport chain to synthesize molecules of ATP through a reaction known as oxidative phosphorylation.

The complete oxidation of one molecule of glucose generates H_2O , CO_2 , and 38 ATP (Schmidt-Nielson, 1997). When oxygen is in short supply, pyruvate is converted to lactic acid. This final product cannot be further catabolised and accumulates in the tissues. When oxygen becomes available, lactic acid is converted back to pyruvate, a process that, in crabs, normally takes place in the hepatopancreas (Chang and O'Connor, 1983).

In crustacean tissues like in vertebrates, when oxygen supply is low, animals must acquire their metabolic energy from anaerobic reactions, namely glycolysis. Glycolysis contributes only 2 ATP per glucose molecule fermented to lactate compared to 38 ATP by oxidative respiration. Therefore, glucose stores must be mobilized to increase the rate of glycolysis to meet energy demands that are not met by oxidative metabolism alone. Prolonged anaerobic metabolism has been evidenced in many crustacean species by increased concentrations of lactate and increased concentrations of glucose in hemolymph (Webster, 1996; Morris and Oliver, 1999; Bergmann et al., 2001). Thus, changes in hemolymph concentrations of glucose and lactate can serve as indicators of metabolic status of crustaceans.

Gas exchange in decapod crustaceans occurs almost exclusively across the surface of the gills (Schmidt-Nielson, 1997). Gills of Tanner crabs are enclosed in the bronchial cavities located dorsally on each side of the animal underneath the carapace. Gill lamellae are small filamentous structures of vascular membranes that serve to facilitate dissolved gas exchange by increasing surface area per gill filament (McMahon and Wilkens, 1983). Crabs maintain a gradient in gas tension between their hemolymph and the media by flushing their gills with water in a unidirectional way. Incurrent water enters through openings at the base of the legs, fills the bronchial chambers, and exits through the mouth. Due to the density of the medium and low oxygen content in water, extra ventilation is often required, such as mechanical pumping with the scaphognathites, exopodite of the second maxilla, to enhance water circulation. The beats generate force

sufficient to move water through the bronchial cavity and across the gills (McMahon and Wilkens, 1983; Schmidt-Nielson, 1997).

As water passes through the gills, dissolved gases diffuse across the gill membrane separating the hemolymph and ambient water (Schmidt-Nielson, 1997). Both O_2 and CO_2 diffuse down their pressure gradient. Thus, O_2 diffuses from the relatively O_2 rich ambient water to the relatively O_2 replete hemolymph, and CO_2 diffuses from hemolymph to water. A countercurrent flow system between hemolymph and water optimizes gradients and efficiency of gas exchange (McMahon and Wilkens, 1983).

Like most advanced crustaceans, Tanner crabs have an open circulatory system and a heart that flushes hemolymph throughout the body. The heart is a single chambered organ with pairs of valved openings (ostias) that lie dorsally within a pericardial cavity, above the gut and between the thoracic gills (Brusca and Brusca, 1990). Oxygen-rich hemolymph at the gills flows to the heart where it is pumped to all body parts. From the heart, major arteries facilitate circulation to vital organs, and empty into an open hemocoel where hemolymph flows freely between tissues. At the tissue and muscle level, oxygen is used in oxidative phosphorylation. Oxygen-poor hemolymph gathers at the gills where it is replenished with O_2 and freed of CO_2 (McLaughlin, 1983; Brusca and Brusca, 1990).

Oxygen carrying capacity of the hemolymph is facilitated by a copper-based respiratory pigment, hemocyanin. Hemocyanin serves to bind, store, and transport oxygen from the site of uptake to the rest of the body (Mangum, 1983). The hemocyanin complex is made of proteins containing copper ions as the site for O_2 -binding. One subunit consists of a large protein with 2 Cu^{2+} ions that reversibly bind one molecule of O_2 . Groups of 2 or 3 subunits constitute one monomer and 6 monomers constitute one hexamer. Polymeric hemocyanin in crustaceans typically consists of 1, 2 or 4 connected hexamers. This respiratory pigment is free in solution, pale blue when oxygenated and colorless when deoxygenated (Mangum, 1983; Withers, 1992).

Animals break down food to produce most of their energy needs through oxidative respiration. Thus, quantifying the amount of oxygen an animal consumes is a measure of its energy metabolism and can be used to express the energy cost of a state such as exercising, stress, or resting (Schmidt-Nielson, 1997). Respirometry is a method to determine rates of oxygen consumption (Cech, 1990; Handy and Depledge, 1999) and has been used in other species to determine changes in metabolic rate associated with temperature, exposure to air, handling and oxygen tension (Morris and Oliver, 1999; Crear and Forteath, 2001; Yaikin et al., 2002). During the course of this study we wanted to evaluate survivorship and performance of Tanner crabs exposed to stressors associated with commercial crab fisheries while monitoring metabolic indices. We measured hemolymph concentrations of glucose and lactate, and oxygen consumption of crabs subjected to air at cold and subfreezing temperatures for different durations to define the range of metabolic responses. These data were used to evaluate performance of crabs following treatments.

Stress physiology

Stress is defined as the response of an organism to a stimulus (stressor) that causes the disruption of the resting physiological state to the extent that chances of survival may be reduced (Barton, 1997). Or put simply, stress is a “threat to or disturbance of homeostasis”. The stress response involves physiological and behavioral changes by an organism to maintain homeostasis in face of a threat. This does not necessarily imply that stress is detrimental to the individual. The response to a stressor is considered an adaptive mechanism that promotes survival. However, if the intensity of the stressor is severe or long lasting and the organism may not escape, the threat becomes detrimental to the individual’s health and well-being. This is also known as a maladaptive or distressed state (Barton, 1997; Barton et al., 2002).

The response of fish to a multitude of stressors such as handling, crowding, disease, water quality, and temperature has been especially well studied, not only for theoretical interests but also for aquaculture and management applications. Wedemeyer et al. (1990) described compensatory responses in terms of primary (neuronal and endocrine pathways), secondary (physiological compensations) and tertiary (whole animal performance) changes. In brief, the primary response involves the stimulation of the central nervous system culminating in the secretion of hormones into the blood stream. This includes stimulation of chromaffin cells in the kidney by the sympathetic nervous system to release catecholamines (epinephrine or adrenaline), and stimulation of the hypothalamus and pituitary that regulate the production and secretion of corticosteroids (mainly cortisol) into circulation. The secondary response refers to alterations of ion concentrations and metabolite levels in plasma and tissue that is caused by physiological adjustments of metabolism, respiration, acid-base balance, hydromineral balance and immune function. The tertiary response concerns whole-animal performance such as growth, reproduction, resistance to diseases, activity and overall survival, which if sufficiently reduced may have effects at the population and community levels (Wedemeyer et al., 1990; Sumpter, 1997; Barton et al., 2002).

These responses to stress are interrelated such that primary responses may directly or indirectly influence secondary and tertiary responses. For example, the release of epinephrine and cortisol serve to mobilize energy stores to cope with the increased energy demands from metabolic activity associated with stress. The main effects of these hormones are to optimize cardiovascular and respiratory functions and regulate energy substrate mobilization and utilization (Wedemeyer et al., 1990). The latter includes the conversion of glycogen into glucose for the more immediate response and replenishment of glycogen stores in the liver via gluconeogenesis for the longer-term maintenance of metabolic substrate. Thus, following an acute stressor, such as handling or brief disturbance, concentrations of plasma glucose increase to provide energy to the animal for the 'fight-or-flight' reaction. In some cases, concentrations of plasma lactate may also

increase as a result of muscular activity associated with stress. Overall, non-essential processes (growth, reproduction) are temporarily halted and resources are redirected towards metabolic processes that would favor survival (Barton et al., 2002).

In invertebrates, the basic characteristics of a stress response are analogous to those found in fish and higher vertebrates. The major glands and production sites involved in stress may be different in such a way that in invertebrates, glands are fewer in number and hold multifunctional roles. However, the same nervous and endocrine responses are implicated, but in a more simplistic way, and the order of the molecular response is analogous (Ottaviani and Franceschi, 1996). Data remains sparse and extensive information on the effects of stressors, responses and molecules concerned in stress are still needed for a broad number of species. In crustaceans, the sinus gland located in the eyestalk stores and releases neuroendocrine hormones, of main interest the crustacean hyperglycemic hormone (CHH; Borst, 2003). CHH and CHH-like peptides secreted from the sinus gland regulate a wide variety of physiological processes such as glucose levels, osmosis, reproduction and molting. The control of hyperglycemic activity has received some attention and recent work has shown that stress such as hypoxia induced the release of CHH and elevated levels of glucose in hemolymph of the crab *Cancer pagarus* (Webster, 1996).

Changes in physiological parameters are characterized by common features whether the stress results from fishing and rearing practices (capture, handling, transport, diseases), environmental alterations (pollution, hypoxia and water quality), or behavioral factors (social hierarchies, biological interactions). However, the type and magnitude of the response and the time it takes an animal to recover depend on the nature, duration and severity of the stressors, the species, and individuals within a species, each with different capacities to respond to stress (Barton, 1997). Additionally, the degree to which an animal responds to multiple stressors is influenced in part by the cumulative physiological effects of the prior stressors (Wedemeyer et al., 1990; Barton, 1997). Since

multiple stressors may have additive or antagonistic effects on animal physiology and behavior, it is important to clearly define the biological system and consider all factors that may affect the stress response.

RESEARCH OBJECTIVES AND CHAPTER ORGANIZATION

This research project was initiated in the fall of 2002 to characterize the physiological responses of Tanner crabs to acute physical stressors modeled on the types of events that can occur in the commercial crab fisheries. Analytical methods used in this project include respirometry and assays of hemolymph constituents to monitor metabolic changes following physical insults such as exposure to air, subfreezing temperatures, and handling. By characterizing these responses to stress and the time to recover, one can better understand the consequences of the physical insult imposed upon crabs captured. The objectives of this research project were to 1) evaluate the impact of various stressors through monitoring physiological changes, and 2) predict the relative vitality and recovery of crabs following stressors by employing physiological indices.

In chapter one, we present background on Tanner crabs and commercial fisheries in Alaska and give a general review on energy metabolism and stress physiology. In chapter two, concentrations of glucose and lactate in the hemolymph of Tanner crabs are determined following various treatments of temperature and air exposure. In chapter three, rates of oxygen consumption are determined before and after Tanner crabs are subjected to subsequent periods of exposure to air at different temperatures. Rates of oxygen consumption were measured with a static respirometry chamber.

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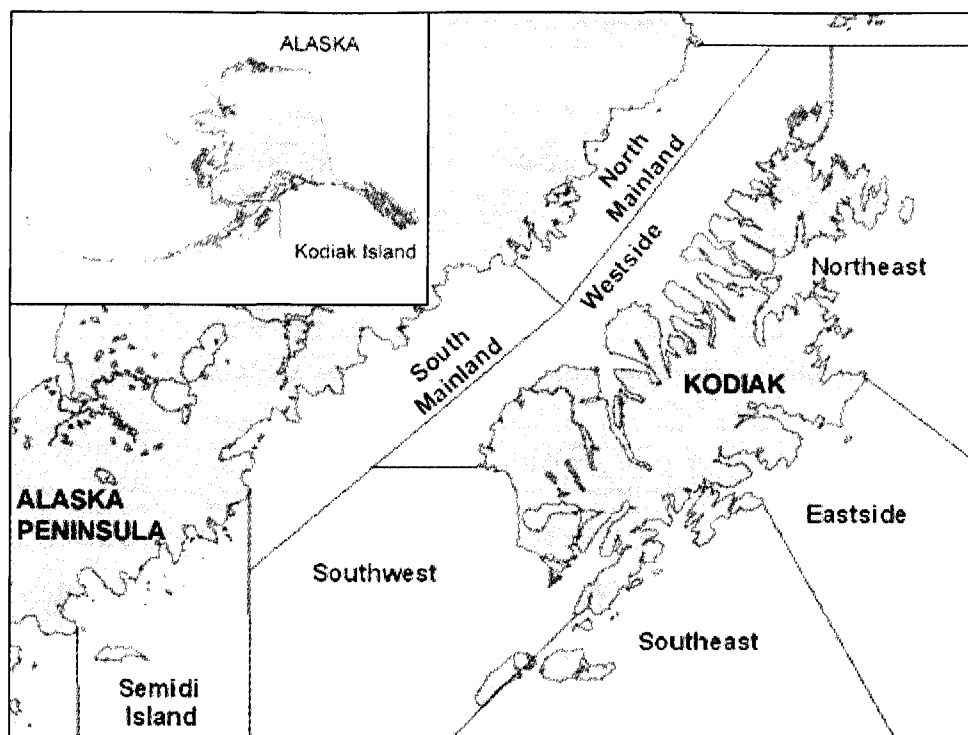


Figure 1.1 Tanner crab fishing sections in the Kodiak District.

This map shows the location of Kodiak Island in relation to Alaska and the fishing sections for Tanner crabs around Kodiak. Maps were reproduced from the Alaska Department of Fish and Game (ADFG).

CHAPTER 2 : EFFECTS OF EMERSION, TEMPERATURE AND HANDLING ON HEMOLYMPH METABOLITES OF TANNER CRABS (*CHIONOECETES BAIRDI*)*

ABSTRACT

In this study, we characterize metabolic responses and subsequent recovery of Tanner crabs following 45 min of air exposure (emersion) at either 8°C or -15°C. Concentrations of glucose and lactate in hemolymph were determined at 30, 60 and 120 min following emersion. Physical responses, including mortality, righting response and limb loss were recorded for up to 7 days of recovery. Concentrations of glucose were significantly lower 30 to 120 min following emersion at -15°C than at 8°C. Concentrations of lactate increased significantly (2 times pre-treatment levels) by 120 min following emersion at -15°C but remained unchanged at intervals following emersion at 8°C. Ninety percent of crabs survived handling and emersion at 8°C, while emersion at -15°C resulted in 71% survival. Among surviving crabs, righting response was significantly lower and limb loss significantly higher among animals emersed at -15°C than those emersed at 8°C throughout the 7 days of recovery. Thus, physiological responses to subfreezing temperature were consistent with low survival, low righting response and increased limb loss.

*El Mejjati S. Y., A. C. M. Oliveira, A. H. Haukenes, and C. L. Buck. 2006. Effects of Emersion, Temperature and Handling on Hemolymph Metabolites of Tanner Crabs (*Chionoecetes bairdi*). Prepared for submission to the Journal of Crustacean Biology.

INTRODUCTION

Commercially harvested and incidentally captured Tanner crabs (*Chionoecetes bairdi*; Rathbun, 1924) in Alaskan waters are subjected to environmental and physical stressors that may impinge on individual survival and fitness. Crabs captured in pots and trawls are exposed to rough handling during the sorting onboard, exposure to air ranging from a few minutes to hours and extreme weather conditions in the winter months with temperatures generally below 0°C. Handling and exposure to low air temperatures is known to induce limb loss, reduce vigor (measured by righting response) and lower survival of crabs (Carls and O'Clair, 1995; MacIntosh et al., 1996; Warrenchuk and Shirley, 2002). Although crabs may survive after capture, physical injuries and stress load may render an individual more vulnerable to predation, less likely to successfully reproduce or result in delayed mortality. Stevens (1990) estimated only 22% survivorship of Tanner crabs inadvertently captured as bycatch in trawls during a commercial fishery for sole. While these data demonstrate a measurable impact on crabs captured, there are little data available describing the physiological responses of Tanner crabs to stressors.

During exposure to air, the gills of aquatic crustaceans may become impaired (Johnson and Uglow, 1985). As a consequence, the demand for oxygen becomes greater than the supply and oxygen requirements are not met. Thus, as the endogenous oxygen supply is depleted an oxygen debt is established and these animals rely on increased anaerobic metabolism to survive (Johnson and Uglow, 1985). In most crustacean species glucose is the primary metabolic substrate and lactate is the most important end product of anaerobic activity (Chang and O'Connor, 1983). Thus, a switch from primarily aerobic to anaerobic metabolism is typically evidenced by an increase in hemolymph lactate and often an increase in hemolymph glucose (Santos and Keller, 1993; Webster, 1996; Morris and Oliver, 1999a; Bergmann et al., 2001; Speed et al., 2001). Swimming crabs (*Liocarcinus depurator*) and squat lobsters (*Munida rugosa*) captured by trawls and then exposed to air for 45 min increase concentrations of both glucose and lactate in

hemolymph compared to controls (Bergman et al., 2001). Similarly, southern rock lobsters (*Jasus edwardsii*) emersed for prolonged periods of time have higher concentrations of glucose and lactate compared to controls (Morris and Oliver, 1999a). There is some evidence that hemolymph levels of glucose can be depleted during prolonged exposure to a stressor, thus, yielding high concentrations of lactate and low concentrations of glucose in hemolymph (Huang and Chen, 2001). Therefore, changes in hemolymph concentrations of glucose and lactate may serve as valuable indicators of metabolic status of crustaceans, although it is not known for Tanner crabs if glucose levels should increase or decrease following exposure to air.

Recovery involves the reversal of physiological disturbances incurred during exposure to air and is complete when anaerobic end products are cleared from the tissues upon re-immersion. Recovery is typically a slow process that takes a few hours for large decapods. Immediately after western rock lobsters (*Panulirus cygnus*) are re-immersed, rates of oxygen consumption increase by 2.5 times the control rates (Crear and Forteach, 2001). These gradually decrease, as do concentrations of both glucose and lactate in the hemolymph, and return to pre-stress levels within 8 hours. Recovery of energy stores is extremely important for animals to cope with further periods of stress. Therefore, crabs might survive stress from capture, but animals returned to the sea or retained in holding tanks may remain vulnerable and susceptible to predation, including cannibalism.

In the present study, concentrations of glucose and lactate in hemolymph of Tanner crabs were determined at various sampling times following exposure to 45 min emersion at 8°C and -15°C in the laboratory. Physical responses such as survival, righting response and limb loss were recorded for up to 7 days post-treatment. We hypothesized that: 1) mortality, righting response and limb loss differ between animals exposed to stress treatments, 2) concentrations of hemolymph glucose and lactate vary among animals subjected to stress treatments and 3) the degree of changes in these constituents is related to the intensity of the stressor.

MATERIALS AND METHODS

Animals and husbandry

Tanner crabs (*Chionoecetes bairdi*) were captured in pots by a commercial fishing vessel in November 2002 from wild stocks near Kodiak Island, Alaska and transported to laboratories at the Kodiak Fisheries Research Center. Approximately 24 crabs were maintained in each rectangular fiberglass tank (1.75 x 1.00 x 0.30 m). Holding tanks were supplied with 100% sand-filtered seawater drawn from 25 meters beneath the surface of Trident Basin at ambient outdoor water temperature (Appendix A). Trials were carried out in December 2002 when the average monthly temperature of seawater was $6.0 \pm 1^\circ\text{C}$. For identification purposes, uniquely coded tags (Floy Tag & Mfg, Inc., Seattle, Washington) were attached to a walking leg of each crab. Carapace width (CW; range 101.1 to 153.5 mm) and chela height (CH) were measured to the nearest 0.1 millimeter at the point of maximum dimension not including the spines, and live wet weights (range 0.32 to 1.32 kg) were recorded to the nearest 0.01 kilogram. Only mature males with a ratio of $\text{CH}/\text{CW} > 0.17$ were used in this project (Stevens et al., 1993). Crabs were fed twice per week ad libitum with majestic squid (*Berryteuthis magister*) supplemented with salmon (*Oncorhynchus spp.*) and halibut (*Hippoglossus stenolepis*) as available. Tanks were cleaned the following day to remove uneaten food and wastes. Crabs were starved for 3 days prior to testing (Paul and Fuji, 1989).

Sampling of hemolymph

Fifty-six crabs were randomly selected and divided into 8 groups ($n = 7$ per group). In one group, crabs were not subjected to any experimental treatment and hemolymph was collected (undisturbed treatment). In a second group, crabs were held out of the water and handled for approximately 5 seconds, returned to seawater in a holding tank, and 75 min later hemolymph was collected (handled treatment). In the remaining 6 groups, crabs

were exposed to air for 45 min either at $8 \pm 0.5^{\circ}\text{C}$ or $-15 \pm 2.1^{\circ}\text{C}$, returned to seawater in a holding tank, and 30, 60 or 120 min after re-immersion hemolymph was collected (emersion treatments). All trials were conducted on the same day (Day 0) and animals were placed in a communal recovery tank. Seven days post-treatment (Day 7) hemolymph was collected for a second time from all surviving crabs. Hemolymph samples were taken from crabs by inserting a 22-gauge needle $1\frac{1}{2}$ inch long with a 5 ml syringe (Med-Vet International, Mettawa, Illinois) through the epimeral line at the rear margin of the carapace (Speed et al., 2001).

Animals were inspected and physical responses were assessed following exposure to a treatment immediately after crab hemolymph was collected on Day 0, the following day (Day 1), and on Day 7. Mortality was recorded and among surviving crabs, limb loss and righting response (ability of a crab to right itself when placed on its back underwater) were measured. Crabs were judged dead when movements of the scaphognathites and locomotory appendices ceased; righting response of a crab was recorded as successful or failure after 5 min (Carls and O'Clair, 1995).

Hemolymph was collected from each crab for the determination of concentrations of glucose and lactate. Prior to analysis, samples of hemolymph (ca. 1ml) were transferred into 1.5 ml polypropylene snap-cap vials and placed in the refrigerator (4°C) overnight to clot. Samples were then centrifuged at 14,000 rpm for 5 min in a microcentrifuge (IEC Micro-MB International Equipment Company, USA). Supernatants were transferred to new 1.5 ml polypropylene vials with Pasteur pipettes and stored at -80°C until assayed (Bergmann et al., 2001).

Glucose assay

Before the glucose assay, hemolymph samples were treated with perchloric acid (PCA) to precipitate proteins out of the solution, using a methodology adapted from Bergmann et al. (2001). Briefly, hemolymph samples were thawed at room temperature and 400 μl of

each sample was mixed with 100 μ l of 8% PCA. The precipitated proteins were separated by centrifugation at 14,000 rpm for 10 min and the supernatants were transferred using Pasteur pipettes to new 1.5 ml polypropylene vials. A 4 M solution of potassium bicarbonate (KHCO_3) was added to achieve a final pH of 7.0 as determined with pH paper. After cooling in a freezer (-20°C) for 5 min, the precipitated potassium perchlorate was separated by centrifugation at 14,000 rpm for 10 min. The supernatants were then transferred with Pasteur pipettes to new 1.5 ml polypropylene vials and stored at -80°C overnight.

The enzymatic microassay for hemolymph glucose was adapted from the methodology described in (Webster, 1996). Enzymatic chromogen reagents were prepared according to instructions provided with Glucose C2 autokits (Wako Chemicals USA, Inc., Richmond, Virginia). Aqueous solutions of glucose standards were prepared at concentrations of 0.0 (blank), 1.0, 2.5, 5.0, 10.0, 20.0, and 40.0 mg dl^{-1} and digested similarly to the hemolymph samples. Hemolymph samples and glucose standards (50 μ l each) were added in triplicate into the wells of a 96-well microplate (Becton Dickinson Labware, Franklin Lakes, New Jersey) with 200 μ l of reagent. To account for inter and intra-plate variation, glucose standards were analyzed on every microplate in replicate. The microplates were placed on an orbital shaker (Troemner, Inc., Thorofare, New Jersey) at 150 rpm for 30 seconds. Samples and standards were then placed in an incubator (model 1555, Sheldon Manufacturing Inc., Cornelius, Oregon) at 34°C for 5 min. To reduce evaporative loss during incubation, microplates were placed on damp paper in a glass container and covered with aluminum foil. Absorbance readings were determined with a microplate reader (Universal Microplate Reader EL800; Bio-Tek Instruments, Inc., Winooski, Vermont) at a wavelength of 515 nm. The average absorbance of the blanks was used to correct the readings of glucose standards and hemolymph samples. Concentrations of glucose were derived from the predictive equation and expressed in mg dl^{-1} of hemolymph.

Lactate assay

The enzymatic microassay for hemolymph lactate was adapted from the methodology described in Chung-Yen et al. (1999). Hemolymph samples were thawed at room temperature. Enzymatic chromogen reagents were prepared according to the instructions provided with the Lactate Instant kit # 735 (Trinity Biotech Inc., Saint Louis, Missouri) and aqueous solutions of lactate standards were prepared at concentrations of 0 (blank), 5, 10, and 20 mg dl⁻¹. Hemolymph samples and lactate standards (2.5 µl each) were analyzed in triplicate with 250 µl of reagent. Samples and standards were homogenized and incubated as per the procedures described in the glucose section. Absorbance readings were determined at a wavelength of 540 nm and the average absorbance of the blank was used to correct the readings of lactate standards and hemolymph samples. Concentrations of lactate were derived from the predictive equation and expressed in mg dl⁻¹ of hemolymph.

Statistical analyses

All statistical tests were performed with STATISTICA 6.1 (StatSoft Inc., Tulsa, Oklahoma). We used a Fisher exact test to determine significant differences in survival, righting response, and limb loss between undisturbed, handled and emersion treatments. Significant differences in concentrations of hemolymph glucose and lactate were determined with the following statistical tests: we used a two-way analysis of variance (ANOVA) to compare crabs exposed to air with temperature and sampling time as factors (Appendix B). Specific differences were determined with Tukey's Unequal N Honestly Significant Difference (HSD). We used a one-way ANOVA to compare all treatment groups on Day 0 and Day 7. The Dunnett's post-hoc test was used to determine significant differences between crabs exposed to each temperature at sampling intervals with undisturbed crabs. When data failed to meet the assumptions of normality and equal variance, a Kruskal-Wallis ANOVA on ranks (H-test) was used. We used a T-test to

compare crabs handled with crabs undisturbed. To take into account the variations in sample size, a type III sum of squares was used to calculate P-values. All differences were considered significant at $P < 0.05$. Weighted means and standard errors (SE) are represented in all graphs and tables for concentrations of glucose and lactate.

RESULTS

Physical responses

The weight ($F_{(7, 48)} = 1.64$; $P = 0.15$) and size ($F_{(7, 48)} = 0.50$; $P = 0.83$) of crabs did not differ significantly between experimental groups prior to any treatment. Crabs that were emersed at -15°C for 45 min displayed diminished vigor throughout the 7 days of recovery as demonstrated by righting response, limb loss and survival data (Table 2.1). Although the percentage survival did not differ significantly among the treatment groups, only 71% of crabs emersed at -15°C survived after 7 days post-treatment compared to 90% of the crabs emersed at 8°C , 100% of the crabs undisturbed and 100% of the crabs handled for 5 seconds. Among the crabs that survived emersion at -15°C , only 33% could right themselves within 5 min by Day 7, which was significantly lower compared to the other treatment groups ($P < 0.05$). Moreover, 80% of these crabs had lost limbs by Day 7, which was significantly greater than the other treatment groups ($P < 0.05$).

Glucose concentrations

The mean concentration of glucose in hemolymph of undisturbed Tanner crabs was $11.15 \pm 1.45 \text{ mg dl}^{-1}$ (Table 2.2). Compared to undisturbed crabs, glucose levels at the intervals sampled following emersion at -15°C were not significantly different ($F_{(3, 23)} = 2.30$; $P = 0.10$). Likewise, glucose levels at the intervals sampled following emersion at 8°C did not differ significantly from pre-treatment levels ($F_{(3, 23)} = 0.78$; $P = 0.52$), but they tended to be greater (1.4 times) at 120 min post-treatment ($15.97 \pm 3.49 \text{ mg dl}^{-1}$). There

was a significant effect of temperature on concentrations of glucose in hemolymph ($F_{(1,36)} = 9.77$; $P = 0.0035$). Concentrations of glucose measured between 30 and 120 min were significantly lower following the -15°C emersion treatment than the 8°C emersion treatment (Figure 2.1).

Handling had a significant effect on short-term changes in concentrations of hemolymph glucose ($T = -2.64$; $d.f. = 11$; $P = 0.023$). Seventy-five minutes after crabs were handled for 5 seconds, the mean concentration of glucose ($17.51 \pm 1.85 \text{ mg dl}^{-1}$) was significantly greater (1.6 times) than pre-treatment levels (Table 2.2). Seven days post-treatment glucose levels of crabs undisturbed, crabs handled for 5 seconds, and crabs emersed within each temperature were similar ($H_{(3,48)} = 6.16$; $P = 0.104$).

Lactate concentrations

The mean concentration of lactate in hemolymph of undisturbed Tanner crabs was $6.12 \pm 1.49 \text{ mg dl}^{-1}$ (Table 2.2). Lactate levels of crabs emersed at -15°C were significantly greater (2.1 times) at 120 min post-treatment ($13.07 \pm 2.44 \text{ mg dl}^{-1}$) compared to undisturbed crabs ($F_{(3,24)} = 3.63$; $P = 0.027$). Although lactate levels at the intervals sampled following emersion at 8°C did not differ significantly from pre-treatment levels ($F_{(3,24)} = 1.47$; $P = 0.25$), they tended to be greater (1.8 times) at 60 min post-treatment ($11.08 \pm 2.73 \text{ mg dl}^{-1}$). There was a significant interaction between temperature and sampling time on concentrations of hemolymph lactate ($F_{(2,36)} = 4.28$; $P = 0.022$). While emersion at -15°C led to increased concentrations of lactate upon re-immersion, lactate levels were not different between 30 and 120 min following emersion at 8°C (Figure 2.1).

Seventy-five minutes after the 5-second handling treatment, concentrations of lactate in hemolymph of crabs ($6.97 \pm 1.70 \text{ mg dl}^{-1}$) did not differ significantly from pre-treatment levels ($T = -0.38$; $d.f. = 12$; $P = 0.71$; Table 2.2). Seven days post-treatment,

lactate levels of crabs undisturbed, crabs handled for 5 seconds and crabs emersed within each temperature were similar ($H_{(3,47)} = 0.1.78$; $P = 0.62$).

DISCUSSION

In laboratory settings, we tested the impacts of handling, emersion and temperature on circulating concentrations of glucose and lactate in hemolymph and subsequent survival of male Tanner crabs. We found that as little as 5 seconds of handling led to increases in levels of hemolymph glucose within 75 min post-treatment. The temperature of emersion impacted both the levels of glucose and lactate in hemolymph observed between 30 and 120 min post-treatment. After re-immersion, crabs exposed to -15°C had significantly lower levels of glucose than crabs exposed to 8°C within the intervals sampled. Concentrations of lactate did not change significantly between 30 and 120 min after exposure to 8°C , whereas they were higher at 120 min following exposure to -15°C than pre-treatment levels.

We assessed crabs for righting response, limb autotomy and mortality on Days 0, 1 and 7 post-treatment to estimate general health and wellness of a crab. The severity of physical responses was dependent on the type of stressor. Crabs emersed at -15°C for 45 min exhibited increased limb autotomy and decreased righting response on Day 0 compared to crabs emersed at 8°C . This trend continued on Days 1 and 7. Limb autotomy describes the severance of an appendage at its autotomy plane and is a normal response to a limb injury among brachyuran crabs (Bliss, 1960; Edwards, 1972). It is common for Tanner crabs to drop a chela or walking leg in response to physical injuries, cold air exposure or other stressors (personal observation; Carls and O'Clair, 1995; MacIntosh et al., 1996). MacIntosh et al. (1996) reported that 85% of Tanner crabs experimentally injured in the legs, autotomized their legs within 24 hours. Emersion at -15°C resulted in delayed mortality (29%) indicating that crabs within this treatment group had failed to recover within 7 days. This is in agreement with other studies, which found that handling

and exposure to low air temperatures induced limb loss, reduced righting response and lowered survival of Tanner and snow crabs (Carls and O'Clair, 1995; MacIntosh et al., 1996; Warrenchuk and Shirley, 2002). Exposure to -15°C was detrimental to Tanner crabs and is consistent with the glucose and lactate responses to subfreezing temperature.

Handling of Tanner crabs for 5 seconds increased concentrations of glucose in hemolymph (1.6 times compared to pre-treatment levels) within 75 min post-treatment. Similarly in other studies, handling has been reported to increase glucose concentrations in crustaceans (Schmitt and Uglow, 1997; Danford et al., 2001). It is likely that the rise in the concentration of hemolymph glucose associated with handling was stimulated by the action of the crustacean hyperglycemic hormone (CHH; Borst, 2003). CHH is known to increase in the circulation of the crab *Cancer pagurus* following emersion, and the increased levels of CHH elicit a sharp increase in concentration of glucose in hemolymph (Webster, 1996). Increased glucose levels are expected to provide a source of readily available energy to the animal to fuel the fight-or-flight response. Handling is typically associated with increased locomotor activity as animals try to escape. Both prawns and lobsters demonstrate escape behaviors such as tail flicking or swimming after disturbance and this is accompanied by increases in rates of respiration (Paterson, 1993; Crear and Fortetooth, 2001).

Handling and 45 min of emersion at 8°C did not result in significant changes in either concentrations of glucose or lactate in hemolymph of Tanner crabs between 30 and 120 min post-treatment compared to pre-treatment levels. It is important to note that while handling Tanner crabs for 5 seconds elicited a stress response as evidenced by an increase in concentrations of glucose in hemolymph, the combination of handling and emersion did not result in elevated concentrations of glucose compared to pre-treatment levels. This is likely influenced by the cumulative effects that multiple stressors may have on stress responses (Barton, 1997). It appears that handling and emersion may have antagonistic effects on glucose concentrations of Tanner crabs. This suggests that Tanner crabs might be metabolic conformers and may be able to reduce their energy expenditure

to tolerate low levels of oxygen. Contingent on the degree of regulation, an oxygen debt does not incur or is reduced and anaerobic compensation may not be necessary (Herreid II, 1980). It is evident that Tanner crabs could tolerate 45 min of emersion at 8°C as seen by 90.5% survival, 100% righting response and 5% limb autotomy after 7 days post-treatment.

It is possible, however, that our sampling strategy missed the peaks of concentrations of glucose and lactate in hemolymph of Tanner crabs. Depending on the species, and on the intensity and the duration of emersion, increases in concentration of hemolymph glucose and lactate could occur during emersion or shortly after re-immersion. For example, concentrations of glucose for the western rock lobster (*Panulirus cygnus*) are not different from pre-treatment levels during 30 min of emersion but increase significantly 1 hour after re-immersion (Crear and Forteach, 2001). Also, the duration that metabolite levels remain elevated varies from minutes to hours depending on the species and the magnitude of the stressor. For swimming crabs (*Liocarcinus depurator*), elevated concentrations of glucose observed during 2 hours of trawling and 1 hour of emersion normalize to pre-treatment levels upon re-immersion and they remain at control levels for 24 hours of recovery (Bergmann et al., 2001). In comparison, American lobsters (*Homarus americanus*) emersed for 50 hours have elevated concentrations of glucose after re-immersion for up to 18 days (Danford et al., 2001). Therefore, it is possible that peaks in concentrations of glucose and lactate in hemolymph of Tanner crabs occurred outside of our sampling range and significant increases might have been detected before 30 min or after 120 min post-emersion at 8°C. This might explain why, even though the increases were not statistically significant, concentrations of glucose tended to be higher (1.4 times pre-treatment levels) 120 min after exposure to emersion at 8°C and concentrations of lactate tended to be higher (1.8 times) 60 min post-treatment.

Handling and 45 min of emersion at -15°C had a significant effect on concentrations of both glucose and lactate in hemolymph of Tanner crabs. Concentrations

of hemolymph glucose of crabs emersed at -15°C were lower between 30 and 120 min post-treatment than that of crabs emersed at 8°C . This is contrary to what one might expect if the stress-load alone was responsible for the modulation in concentration of glucose. However, temperature has effects on rates of reaction following a Q_{10} relationship (Davenport, 1992). In crustaceans, a 10°C drop in temperature typically reduces rates of respiration by 2 to 4 times (Crear and Forteach, 2001). It is well known that cold temperatures slow down rates of respiration and cell metabolism (Morris and Oliver, 1999a, b; Danford et al., 2001; Huang and Chen, 2001). Chilled southern rock lobsters (*Jasus edwardsii*) that were emersed and packaged on ice had lower rates of oxygen consumption and reverted to anaerobiosis a day later than non-chilled lobsters. Hyperglycemia was achieved after 1 hour of emersion in chilled lobsters compared to 2 min in non-chilled lobsters (Morris and Oliver, 1999a). In our study, it is likely that exposure to -15°C slowed rates of reaction, causing both slower mobilization and utilization of glucose upon re-immersion compared to exposure to 8°C . Thus, at the lower temperature there was delayed and diminished amplitude of response even though the intensity of the stressor was greater.

In addition to the effects of cooling, damage to the respiratory structures and tissues likely occurred from exposure to subfreezing temperature for extended duration. In a preliminary study, Tanner crabs were exposed to air at -15°C for up to 100 min and leg and core temperatures were measured with a temperature probe at 2 min intervals (Appendix C). As little as 15 min of exposure to -15°C caused leg temperatures to drop below 0°C , and after 30 min of exposure, legs were likely frozen. Core temperatures decreased slowly and after 30 min they were close to 0°C . After 45 min of exposure crabs were likely supercooled, and although animals were not frozen, some structures like gills and legs were probably frozen. Results from this study indicate that emersion at -15°C for 45 min was detrimental as seen by increased mortality, increased limb loss and reduced righting response throughout the 7 days of observation.

As with glucose, concentrations of lactate varied with temperature of treatment. Emersion for 45 min at -15°C led to increased concentrations of lactate 120 min post-treatment compared to pre-treatment levels. This is probably not due to an oxygen debt acquired during emersion since the temperature of exposure expected to generate the greater energy debt is 8°C and there was no accumulation of lactate at 8°C over the specific sampling intervals. A more likely cause for the increase in concentration of lactate following exposure to -15°C is an incurred oxygen debt due to an impaired respiratory apparatus of the crabs from freezing. Upon re-immersion, crabs apparently could not extract oxygen from the water and necessarily relied to a greater extent on anaerobic metabolism. This would explain why increases in lactate concentrations were not detected until 120 min after the emersion treatment. Additionally, it is likely that the effects of temperature and Q_{10} also contributed to the delay in accumulation of lactate. Q_{10} effects on energy mobilization and utilization likely diminished the rates of formation and appearance of lactate in the hemolymph of Tanner crabs emersed for 45 min at -15°C as compared to those emersed at 8°C .

In conclusion, these data illustrate the time course of changes in concentrations of hemolymph glucose and lactate following exposure to handling, 45 min of emersion and severe subfreezing temperature in a high latitude crustacean. Emersion for 45 min at -15°C proved to be extremely harmful to Tanner crabs, whereas a similar treatment at 8°C induced fewer injuries and lower rates of mortality. Also, the intensity of the stressor corresponded with lower levels of glucose and increased levels of lactate. Thus, physiological responses to subfreezing temperature were consistent with low survival, low righting response and increased limb loss. The physical stressors are modeled after the type of events that occur in the commercial crab fisheries. Although crabs may survive capture, they are likely compromised and vulnerable to further periods of stress. Decreased viability of crabs returned to sea may have an impact on future recruitments.

Therefore, any changes in the means of harvest that could increase survivorship of bycatch would benefit sustainable fisheries.

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Table 2.1 Percentage of Tanner crabs that survived (S), righted (R), and lost limbs (L).

Physical responses were assessed immediately following exposure to a treatment (Day 0), the following day (Day 1), and 7 days post-treatment (Day 7), among crabs not subjected to any treatment (undisturbed), among crabs that were handled for 5 seconds (handled), and among crabs that were emersed at 8°C or -15°C. Distinct letters show significant differences within each column at $P < 0.05$.

Treatment	Day 0				Day 1				Day 7			
	n	% S	% R	% L	n	% S	% R	% L	n	% S	% R	% L
Undisturbed	7	100.0	100.0 ^a	14.3 ^{ab}	7	100.0	85.7 ^{ab}	--	7	100.0	100.0 ^a	14.3 ^a
Handled	7	100.0	100.0 ^a	0.0 ^{ab}	7	100.0	100.0 ^a	--	7	100.0	100.0 ^a	0.0 ^a
Emr at 8°C	21	100.0	95.0 ^a	0.0 ^a	19	90.5	100.0 ^a	--	19	90.5	100.0 ^a	5.3 ^a
Emr at -15°C	21	100.0	5.0 ^b	19.1 ^b	18	85.7	55.6 ^b	--	15	71.4	33.3 ^b	80.0 ^b

n sample size; Emr emersion

Table 2.2 Average concentrations \pm SE of glucose (mg dl^{-1}) and lactate (mg dl^{-1}) in the hemolymph of Tanner crabs.

Glucose and lactate levels were estimated immediately following exposure to a treatment (Day 0) and 7 days post-treatment (Day 7), among crabs not subjected to any treatment (undisturbed), among crabs that were handled for 5 seconds (handled), and among crabs that were emersed at 8°C or -15°C . Means with an asterisk (*) indicate a significant difference from that of crabs that were undisturbed within each column at $P < 0.05$.

Treatment	Day 0			Day 7		
	n	Glucose	Lactate	n	Glucose	Lactate
Undisturbed	7	11.15 ± 1.45	6.12 ± 1.49	7	9.60 ± 0.94	6.02 ± 1.28
Handled	7	$17.51 \pm 1.85^*$	6.97 ± 1.70	7	12.91 ± 2.28	5.92 ± 1.21
Emersion at 8°C	21	14.61 ± 1.32	9.25 ± 1.11	19	10.34 ± 0.76	7.31 ± 0.71
Emersion at -15°C	21	9.50 ± 0.97	9.01 ± 1.16	15	11.82 ± 1.19	5.97 ± 1.01

n sample size

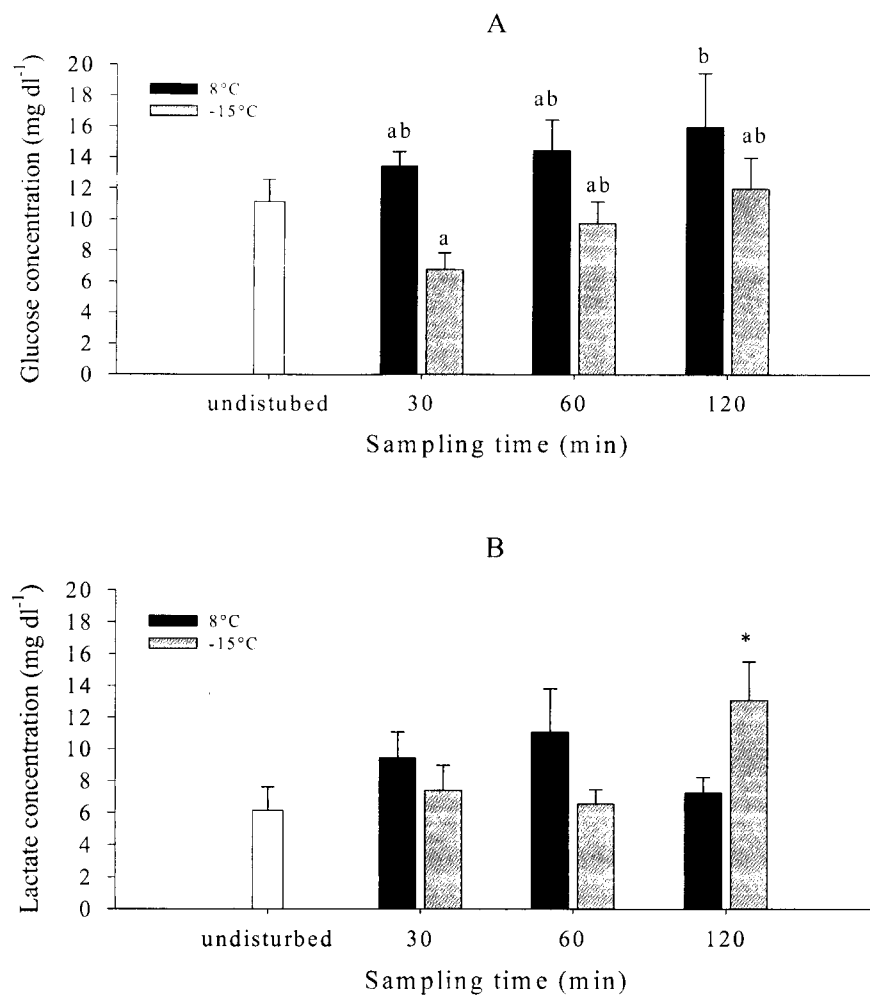


Figure 2.1 Concentrations of (A) glucose and (B) lactate in hemolymph of Tanner crabs. Glucose and lactate levels were estimated at 30, 60 and 120 min after crabs were exposed to air at 8°C or -15°C and among crabs not subjected to any treatment (undisturbed). Vertical bars represent means (+ SE). Means with distinct letters indicate significant differences among crabs that were emerged at $P < 0.05$. Means with an asterisk (*) show a significant difference from that of crabs that were undisturbed at $P < 0.05$.

CHAPTER 3 : EFFECTS OF EMERSION, TEMPERATURE AND HANDLING ON THE OXYGEN CONSUMPTION OF TANNER CRABS (*CHIONOECETES BAIRDI*) *

ABSTRACT

In this study we characterize metabolic responses and recovery of Tanner crabs following 15, 30 or 45 min of air exposure (emersion) at 8°C or -15°C. Rates of mass specific oxygen consumption (VO_2) were determined before and after treatments and rates of mortality were recorded throughout the experiment. VO_2 increased immediately following 15, 30 and 45 min emersion at 8°C (on average 1.5 times pre-treatment levels), while 45 min emersion at -15°C resulted in depressed VO_2 (0.3 times pre-treatment levels). All crabs survived emersion at 8°C and VO_2 returned to pre-treatment levels within 12 hours. These animals responded to a second emersion treatment by increasing VO_2 upon re-immersion. In comparison, crabs emersed at -15°C for 30 and 45 min were clearly compromised. Crabs survived 30 min exposure, but VO_2 did not increase following the second emersion treatment. Exposure for 45 min led to depressed VO_2 post-treatment for up to 12 hours and low survival rates (25%) following the second emersion treatment. Emersion for 15 min at -15°C did not cause the same amount of damage to crabs as 30 or 45 min exposure. These results indicated that the longer durations of emersion at -15°C were the greater insults to the recovery and survival of Tanner crabs and low percentage survival corresponded with depressed VO_2 .

* El Mejjati S. Y., A. C. M. Oliveira, A. H. Haukenes, and C. L. Buck. 2006. Effects of Emersion, Temperature and Handling on the Oxygen Consumption of Tanner Crabs (*Chionoecetes bairdi*). Prepared for submission to the Journal of Crustacean Biology

INTRODUCTION

The number of Tanner crabs (*Chionoecetes bairdi*; Rathbun, 1924) that are caught as bycatch in the Alaskan commercial fisheries is substantial. During the 1994 Bering Sea snow crab fisheries, approximately 9 million Tanner crabs were caught and released (Tracy, 1995). These captured animals are subjected to physical and environmental conditions while on deck that may be lethal to individuals. Crabs are often exposed to air and extreme temperatures for minutes to hours depending on the fishery (pot versus trawl) and the number of crabs caught (Stevens, 1990; Warrenchuk and Shirley, 2002a). In addition, rough handling onboard as well as contact with heavy gear can cause severe injuries. Snow crabs (*Chionoecetes opilio*) and Tanner crabs exposed to air at subfreezing temperatures demonstrated increased limb loss, reduced vigor (measured by righting response) and lower survival (Warrenchuk and Shirley, 2002b; Carls and O'Clair, 1995). Although crabs may survive after capture, physical injuries and stress load may render an individual more vulnerable to predation, less likely to successfully reproduce or result in delayed mortality. Stevens (1990) estimated only 22% survivorship of Tanner crab bycatch captured during a commercial sole trawl.

Aquatic animals cannot effectively breathe in air and rates of oxygen consumption decrease due to decreased gas exchange. The degree of the drop in oxygen consumption is species specific and is more pronounced in subtidal than intertidal crabs (O'Mahoney and Full, 1984; Albert and Ellington, 1985). This is primarily due because they lack rigid gill structures that provide more resistance to clumping of gill lamellae. Upon exposure to air, the gills of subtidal crabs collapse decreasing the surface area for gas exchange (Johnson and Uglow, 1985; deFur et al., 1988). Rates of oxygen consumption of the sub-littoral crab, *Liocarcinus puber*, in air are only 19% of the submerged values compared to 58% for the intertidal crab, *Carcinus maenas* (Johnson and Uglow, 1985). Rates of oxygen consumption of the aquatic crab, *Callinectes sapidus*, exposed to air are reduced to 1/3 of the submerged levels (O'Mahoney and Full, 1984),

and similarly, rates of oxygen consumption of emersed southern rock lobsters, *Jasus edwardsii*, are reduced to 30% of the submerged values (Morris and Oliver, 1999a). Tanner crabs are subtidal and are rarely if ever exposed to air naturally (Jadamec et al., 1999). It is unlikely that this species is adapted to long periods of emersion.

Because the respiratory system is impaired during emersion, oxygen uptake is reduced and normal oxygen requirements are not met. When the demand for oxygen becomes greater than the available supply, the animal incurs an oxygen debt (Johnson and Uglow, 1985; Crear and Forteath, 2001a). Animals may be able to compensate for this oxygen debt by decreasing their total metabolic rate. Herreid II (1980) described respiratory responses of different invertebrates during hypoxia that he characterized as two extreme strategies. Metabolic regulators maintain VO_2 independently of the oxygen tension. In contrast, metabolic conformers are dependent on oxygen availability and VO_2 drops with oxygen tension. Some species can adopt both strategies (Herreid II, 1980). For example, the western rock lobster, *Panulirus cygnus*, was able to maintain an elevated VO_2 down to a critical tension of 47 torr at which point VO_2 decreased linearly with decreasing oxygen tension (Crear and Forteath, 2001a). Therefore, by reducing their energy expenditure during emersion, conformers can reduce their oxygen debt and, depending on the degree of regulation, they may or may not rely on increased anaerobic activity (see review in chapter 2). An oxygen deficit is rectified by increased rates of oxygen consumption upon re-immersion and recovery is characterized by O_2 balance, restoration of energy stores and elimination of metabolic wastes. The post-emersion increase in oxygen consumption should be equal to the debt (Crear and Forteath, 2001b).

Environmental physiologists have identified a number of quantitative, biochemical markers of stress in marine organisms (Morgan and Iwama, 1997; Handy and Depledge, 1999; Dahlhoff, 2004). The premise is that stress is a homeostatic imbalance and adjustments in the rates of biochemical and physiological processes are necessary to ameliorate the imbalance imposed by the stressor. These adjustments incur a

metabolic cost. Therefore, given the interrelationships between stress, metabolism and aerial exposure, metabolic rate of marine organisms has proven an excellent indicator of physiological condition (Childress and Seibel, 1998).

In this study we focus on metabolic responses of Tanner crabs to disturbances normally encountered during capture and post-capture conditions. Rates of oxygen consumption were determined using a static respirometry system in the laboratory both before and after crabs were exposed to periods of emersion ranging from 15 to 45 min at either 8°C or -15°C. Following a recovery period, all surviving crabs were exposed to a uniform stressor (emersion at -15°C for 10 min). This enabled us to determine the effects of a subsequent exposure to a stressor on rates of oxygen consumption. Mortality rates were recorded throughout testing. We hypothesized that 1) rates of mortality differ between animals exposed to stress treatments, 2) rates of oxygen consumption vary among animals subjected to stress treatments and 3) the degree of changes in the rates of oxygen consumption is related to the intensity of the stressor.

MATERIALS AND METHODS

Animals and husbandry

Tanner crabs (*Chionoecetes bairdi*) were captured in pots by a commercial fishing vessel in November 2002 from wild stocks near Kodiak Island, Alaska and transported to laboratories at the Kodiak Fisheries Research Center (KFRC). Approximately 24 crabs were maintained in each rectangular fiberglass tank (1.75 x 1.00 x 0.30 m). Holding tanks were supplied with 100% sand-filtered seawater drawn from 25 meters beneath the surface of Trident Basin at ambient outdoor water temperature (Appendix A). Respirometry trials were carried out between May and June 2003 (6 months after crabs were captured) when average monthly temperatures of seawater varied between $7.2 \pm 0.4^\circ\text{C}$ and $8.2 \pm 0.28^\circ\text{C}$, respectively. For identification purposes, uniquely coded tags

(Floy Tag & Mfg, Inc., Seattle, Washington) were attached to a walking leg of each crab. Carapace width (CW; range 105.7 to 153.5 mm) and chela height (CH) were measured to the nearest 0.1 millimeter at the point of maximum dimension not including the spines, and live wet weights (range 290 to 1158 g) were recorded to the nearest gram. Only mature males with a ratio of $CH/CW > 0.17$ were used in this project (Stevens et al., 1993). Crabs were fed twice per week ad libidum with majestic squid (*Berryteuthis magister*) supplemented with salmon (*Oncorhynchus spp.*) and halibut (*Hippoglossus stenolepis*) as available. Tanks were cleaned the following day to remove uneaten food and wastes. Crabs were starved for 3 days prior to testing (Paul and Fuji, 1989).

Respirometry chambers

For the respirometry trials, crabs were placed in static respirometry chambers similar in design to that described by Cech (1990). Individual chambers consisted of a polycarbonate container (66 x 46 x 15 cm) with a clear Lexan® cover, to which an oxygen probe (YSI Incorporated, Yellow Springs, Ohio) and flushing tubes were fitted. Small holes at opposite corners served to release pressure and a rectangular aperture (20 x 23 cm) facilitated handling of crabs. A second Lexan® sheet (40 x 33 cm) covered the aperture and was held closed with plastic bolts. Water source and quality in the chamber were the same as that in holding tanks. A small electric pump mixed water inside the chamber to ensure homogeneity. The chamber was $\frac{3}{4}$ submerged in a holding tank with flowing seawater to maintain constant temperature. When crabs were not being tested, fresh seawater continuously flowed through the chambers. Prior to collecting respirometry measurements, all air was removed and the chamber was sealed with vacuum grease (Dow Corning Corporation, Midland, Michigan).

Measurement of rates of oxygen consumption

During respirometry trials, dissolved oxygen concentration (DOC), temperature and conductivity were recorded every minute for a sampling period (SP) of 1-2 hours

following equilibration of the oxygen probe (15 min). At the end of all respirometry trials for each individual, the animal was weighed and its rate of mass specific oxygen consumption was calculated as follows (Cech, 1990):

$$VO_2 = (DOC_b - DOC_a) * V / T / m$$

Where VO_2 is the rate of oxygen consumption per unit mass ($mgO_2 g^{-1} hr^{-1}$), DOC_a is the initial dissolved oxygen concentration ($mgO_2 L^{-1}$), DOC_b is the final dissolved oxygen concentration ($mgO_2 L^{-1}$), V is the volume (L) of water in the chamber, T is the time interval (hr) and m is the wet weight (g) of the crab.

After individual crabs were removed from the chambers, DOC was determined in chambers without crab for 1 hour to assess biological activity. Biological activity averaged $8.4 \times 10^{-4} mgO_2 L^{-1} min^{-1}$ and this value was used to correct oxygen consumption rates of crabs (Cech, 1990).

Changes in VO_2 following the emersion treatments

In the closed chambers, seawater temperatures ranged between 7.13 and 9.54°C and did not vary more than 1°C within each sampling period. Fifty-two crabs were randomly selected for this experiment. At the start of the respirometry trials, crabs were placed in individual chambers and allowed to acclimate for 12 hours. The duration of acclimation was based on preliminary tests that established the time required for crab VO_2 to return to baseline after handling (El Mejjati, data not shown). Prior to exposure to any treatment, standard metabolic rate (SMR) was determined for each crab ($n = 52$; Figure 3.1). Thereafter, crabs were randomly divided into 6 groups ($n = 8-10$). Each group was subjected to a primary stressor consisting of one of the following 6 treatments: emersion in a cold room at $8 \pm 0.5^\circ C$ for 15, 30 or 45 min or emersion in a chest freezer at $-15 \pm 2.1^\circ C$ for 15, 30 or 45 min. Durations of exposure were based on preliminary tests that

established 60 min as the lethal limit for -15°C (El Mejjati, data not shown). After crabs were re-immersed in chambers, VO_2 was determined in sampling periods immediately following the 1st stressor (SPI) and 12 hours following the 1st stressor (SPII). All crabs were removed from chambers and those crabs that survived were exposed to a uniform 2nd stressor: emersion at $-15 \pm 2.1^{\circ}\text{C}$ for 10 min. After crabs were re-immersed in chambers, VO_2 was determined in sampling periods immediately following the 2nd stressor (SP III) and 12 hours following the 2nd stressor (SP IV). Mortality was recorded for all individuals throughout the experiment and expressed as percentage survival. Crabs were judged dead when movements of the scaphognathites and locomotory appendages ceased (Carls and O'Clair, 1995).

Correction for probe drift

Before collecting metabolic measurements, the probes were calibrated according to the procedures provided by YSI (YSI Incorporated, Yellow Springs, Ohio). Thereafter, the probe was placed in oxygen-saturated seawater and dissolved oxygen concentration (DOC) was measured before and after the experimental trials. When the percentage change in DOC exceeded the instrumental error ($\pm 2\%$) as reported by the manufacturer, a rate of drift ($\text{mgO}_2 \text{ L}^{-1} \text{ min}^{-1}$) was calculated as the change in DOC per unit time (drift was linear; Appendix D). The rate of drift was used to correct oxygen consumption rates of crabs for the appropriate sampling period (SP). Among all SP ($n = 248$), 20% were affected by probe drift and within each drift, the change in DOC was less than 15%. In 7.3% of all SP, DOC did not stabilize within 15 min in oxygen-saturated seawater and these data were excluded from statistical analysis.

Statistical analyses

All statistical tests were performed with STATISTICA 6.1 (StatSoft Inc., Tulsa, Oklahoma). We used a Fisher exact test to determine significant differences in survival

between emersion treatments within each sampling period. To determine significant differences in VO_2 between emersion treatments within each sampling period, we used a two-way analysis of variance (ANOVA) with temperature and duration of emersion as factors (Appendix E). To determine significant differences in VO_2 between sampling periods within each emersion treatment, we used a one-way repeated measures (RM) ANOVA. Specific differences were determined with post hoc tests using Tukey's Unequal N Honestly Significant Difference (HSD). When data failed to meet the assumptions of normality and equal variance, a Kruskal-Wallis ANOVA on ranks (H-test) or Friedman RM ANOVA on ranks was used. To take into account the variations in sample size, a type III sum of squares was used to calculate P-values. All differences were considered significant at $P < 0.05$. Weighted means and standard errors (SE) are represented in all graphs and tables for rates of oxygen consumption.

RESULTS

Percentage survival of Tanner crabs

The weight ($F_{(2, 46)} = 0.647$; $P = 0.528$) and size ($F_{(2, 46)} = 0.204$; $P = 0.816$) of crabs did not differ significantly between experimental groups prior to any treatment. A regression analysis showed no linear relationship ($P = 0.344$; $r^2 = 0.02$) between log of mass specific SMR ($\text{mgO}_2 \text{ g}^{-1} \text{ hr}^{-1}$) and log of body mass (g). Emersion for 45 min at -15°C resulted in decreased survival of crabs over time (Table 3.1). Within this treatment, the percentage of crabs that survived in SP III (37.5%) and SP IV (25.0%) was significantly lower compared to that in all other emersion treatments, which had 100% survival throughout the experiment ($P < 0.05$). Accordingly, VO_2 of crabs emersed for 45 min at -15°C were excluded from statistical analyses in SP III and SP IV due to low sample size.

VO₂ of Tanner crabs between treatment groups

SMR did not differ significantly among experimental groups ($F_{(2, 41)} = 0.14$; $P = 0.873$) and averaged $0.039 \pm 0.015 \text{ mgO}_2 \text{ g}^{-1} \text{ hr}^{-1}$ ($n = 47$; Figure 3.2). In SPI (immediately after the 1st stressor), there was a significant interaction between temperature and duration of emersion on VO₂ ($F_{(2, 41)} = 3.83$; $P = 0.030$). While VO₂ of crabs were similar among durations of emersion at 8°C, VO₂ decreased as the duration of emersion at -15°C increased. Crabs emerged at -15°C for 30 and 45 min had the lowest VO₂, compared to all other treatment groups. In SPII (12 h after the 1st stressor), there was a significant effect of temperature on VO₂ ($F_{(1, 41)} = 8.36$; $P = 0.006$). Crabs emerged at -15°C had significantly lower VO₂ overall than crabs emerged at 8°C. In SPIII (immediately after the 2nd stressor), crabs emerged at -15°C for 30 min had the lowest VO₂ compared to all other treatment groups ($F_{(4, 36)} = 4.39$; $P = 0.005$). In SPIV (12 h after the 2nd stressor), VO₂ of surviving crabs were similar among treatment groups ($F_{(4, 37)} = 0.63$; $P = 0.643$).

VO₂ of Tanner crabs between sampling periods (SP)

VO₂ of crabs varied among SP depending on the intensity of the treatment (Figure 3.3). VO₂ of crabs that were emerged at 8°C followed a similar pattern for all durations of exposure. VO₂ increased immediately after the 1st and 2nd stressors (SPI and SPIII, respectively) and returned to baseline within 12 hours post-treatment (SPII and SPIV). Since VO₂ of all crabs emerged at 8°C were not significantly different between durations of exposure (Figure 3.2), data were pooled. The average VO₂ for all three durations increased significantly (1.4 times) in SPI and (1.5 times) in SPIII from SMR ($F_{(4, 80)} = 17.12$; $P = 0.0000$). In comparison, VO₂ of crabs that were emerged at -15°C varied among the different experimental durations. Crabs emerged at -15°C for 15 min had significantly lower VO₂ 12 hours after the 1st and 2nd stressors (SPII and SPIV) compared to SMR ($F_{(4, 20)} = 7.40$; $P = 0.0008$). Crabs emerged at -15°C for 30 min had significantly lower VO₂ in SPI than in SPIII ($\chi^2 = 15.56$; $d.f. = 4$; $P = 0.004$). Although it was not

statistically different, VO_2 tended to be lower (0.6 times) in SPI compared to SMR. Crabs emersed at -15°C for 45 min had significantly lower VO_2 (0.3 times) in SPI and (0.4 times) in SPII compared to SMR ($F_{(2, 10)} = 7.80$; $P = 0.009$).

DISCUSSION

This study examines the metabolic responses of wild caught male Tanner crabs following periods of handling and emersion at 8°C and -15°C in terms of rates of oxygen consumption and survival in captivity. Results indicate that VO_2 varies with both temperature and duration of emersion. After re-immersion, crabs exposed to 8°C had higher VO_2 than crabs exposed to -15°C . Also, VO_2 increased following all durations of emersion at 8°C , while VO_2 decreased as the duration of emersion at -15°C increased. Except for crabs emersed at -15°C for 45min, all animals survived 12 hours after the treatments and VO_2 returned to baseline values. Those crabs emersed at -15°C for 45 min had depressed rates of oxygen consumption post-treatment up to 12 hours and low survival rates.

As hypothesized, handling and emersion at 8°C led to an increase in VO_2 upon re-immersion. This might be the metabolic cost associated with the oxygen debt that was incurred, even though we did not measure changes in rates of respiration during air exposure. Metabolic rates of reaction increase in crustaceans at higher body temperatures (Davenport, 1992). Yet animals cannot effectively extract oxygen from air because of its deleterious effects on gills (Johnson and Uglow, 1985; deFur et al., 1988). Thus, in most crustaceans, exposure to air is accompanied by a decrease in oxygen consumption. The oxygen consumption rates of rock lobsters (*Jasus edwardsii*) exposed to air for 30 min is only 30% that of the submerged values (Morris and Oliver, 1999a). Similarly, it seems reasonable to hypothesize that Tanner crabs could not maintain VO_2 in an air environment and accumulated an oxygen deficit.

It is important to note that all crabs emersed at 8°C responded similarly regardless of the duration of exposure. One might expect that the longer animals are out of water the greater the oxygen debt and animals with a greater oxygen debt would have a higher VO_2 one hour following re-immersion. There is a possibility that the peak in VO_2 occurred after 1 hour of re-immersion, and consequently our sampling methodology missed the increase. Another potential explanation is that the magnitude of the debt incurred is described by both the magnitude of the increase in VO_2 and the recovery pattern of animals. If animals can only increase VO_2 to a plateau at a given temperature, it is the duration that VO_2 remains elevated that will allow them to repay the debt. Thus, animals that incur a larger oxygen deficit might maintain a higher VO_2 for a longer duration than those with a lesser deficit. We sampled for VO_2 only immediately after emersion and 12 hours later; therefore, we cannot draw any firm conclusion concerning the recovery pattern of these animals without the data points in between these. Nonetheless, there is evidence that metabolic recovery is a slow process and rates of oxygen consumption do not immediately return to baseline upon re-immersion. Typically, recovery takes about 8 hours in large decapods, such as for the western rock lobster (*Panulirus cygnus*), and within this time the oxygen debt acquired during emersion is repaid, lactate is metabolized and glycogen stores are replenished (Crear and Forteach, 2001b).

Conversely, VO_2 of Tanner crabs could have increased mainly in response to handling. Tanner crabs, like other decapod crustaceans, might be able to tolerate short periods of emersion by reducing their rates of oxygen consumption depending on oxygen availability and thereby conserving energy (Herreid II, 1980). If Tanner crabs are metabolic conformers and could reduce their metabolism during air exposure, an oxygen debt would be reduced and handling could be primarily responsible for the elevated VO_2 . This would explain why there is no correlation between oxygen debt and the duration of exposure following emersion at 8°C.

There is some evidence that handling induced an increase in VO_2 following air exposure at 8°C. One major effect of handling is that it stimulates an increase in locomotor activity of animals, causing an increase in respiration rates (Winkler, 1987; Paterson, 1993). Similarly, a change in the activity level of Tanner crabs following handling and emersion was observed. Upon return to the respirometry chambers, crabs that were exposed to 8°C were agitated and aggressive, while crabs exposed to -15°C were less responsive (personal observation). Furthermore, concentrations of glucose in the hemolymph of Tanner crabs increased after handling for 5 seconds (see Chapter 2). These data indicate that handling Tanner crabs during the 8°C emersion treatment elicited a stress response as evidenced by increased respiration rates. One would expect handling to have similar effects under all emersion treatments. However, this was not the case following emersion at -15°C possibly because at lower temperatures, rates of biochemical reaction are further depressed and handling elicits a lesser metabolic response. Tanner crabs could also have been compromised and less capable of responding to handling and emersion stress after exposure to the extended durations (30 and 45 min) at -15°C, as discussed in the following paragraphs.

Although the effects of handling and emersion could not be separated, it is important to note that the methodology was standardized at both temperatures and that there was a temperature effect on VO_2 between experimental groups. Metabolic responses differed significantly between the 8°C and -15°C emersion treatments and these treatments also caused differential survivorship of the animals. In addition, duration of exposure had a significant effect on VO_2 of crabs at the lower temperature.

On average, VO_2 was significantly lower immediately following the -15°C emersion treatment compared to the 8°C emersion treatment. This was to be expected as a drop in temperature reduces rates of cellular reactions in a Q_{10} dependent fashion (Davenport, 1992). In crustaceans, oxygen consumption decreases exponentially as the temperature decreases, typically at a rate of 2 to 4 (Crear and Forteach, 2000, 2001a, b). In addition, the affinity of hemolymph for oxygen increases at low temperatures,

resulting in decreased oxygenation to the tissues, and subsequently in decreased muscle activity (Withers, 1992; Morris and Oliver, 1999b). These combined effects contribute to a reduction in whole animal metabolism and subsequently rates of oxygen consumption are reduced. It is well known in the fishing industry that pre-cooling prolongs survivorship of animals by reducing their metabolism, and thus is commonly used during live transport of crustaceans (Paterson, 1993; Samet et al., 1996; Morris and Oliver, 1999a; Crear and Forteach, 2000). In this study, Tanner crabs were lethargic and less responsive to disturbances (personal observations) following emersion at -15°C , and this was accompanied by a decrease in respiration rate.

Crabs emersed at -15°C for 15 min showed a peculiar trend in VO_2 . Immediately following the 1^o and 2^o stressors, VO_2 did not differ from pre-treatment levels, but it decreased significantly 12 hours later (Figure 3.3). The average SMR for this experimental group tended to be higher than that of other groups, although not significantly (Figure 3.2). This could be a spurious result and in order to look at this data differently, all SMR were pooled to minimize experimental artifact. Thus, we used the average SMR of all experimental groups to calculate the change in VO_2 at each sampling period (expressed as % SMR; Figure 3.4). Given this new look at the data it appears as though VO_2 followed a similar pattern as seen for the 8°C animals. VO_2 increased immediately after the 1^o and 2^o stressors and returned to baseline 12 hours after the treatments.

There was a significant effect of duration of emersion following exposure to -15°C . VO_2 of Tanner crabs emersed at -15°C decreased from SMR but only when the exposure time was greater than 30 min. Exposure for 15 min led to an increase in VO_2 from baseline by 18%, while exposure for 30 and 45 min resulted in a decrease in VO_2 by 41 and 69%, respectively (Figure 3.4). It takes time for a crab to cool and, as it is cooling, metabolic processes are probably slowing because of Q_{10} effects on rates of reaction. Thus, after 15 min of emersion at -15°C , Tanner crabs had not cooled appreciably as

compared to 30 or 45 min of exposure. It is clear that the longer the duration of exposure, the greater the impact of temperature and "an extreme temperature might be tolerated for several minutes but may be lethal if continued for several hours" (Schmidt-Nielson, 1997).

Exposure to subfreezing temperature for extended duration not only reduces metabolism but can cause damage to the animals. It is likely that after 45 min of exposure to -15°C , respiratory structures of Tanner crabs were frozen. The longer animals are exposed to temperatures well below the ice nucleation point of tissues, the greater the chances of freezing them (Davenport, 1992). In a preliminary study, Tanner crabs were exposed to air at -15°C for up to 100 min and leg and core temperatures were measured with a temperature probe at 2 min intervals (Appendix C). As little as 15 min exposure to -15°C caused leg temperatures to drop below 0°C and after 30 min exposure, legs were likely frozen. Core temperatures decreased slowly and were close to 0°C after 30 min exposure at -15°C . After 45 min exposure crabs were likely supercooled, and although animals were not frozen, some structures like gills and legs were probably frozen. Apparently, Tanner crabs were unable to recover from this temperature and duration of emersion as evidenced by the high mortality rates (75%). In comparison, crabs exposed for 30 min could recover, suggesting that these animals did not incur the same amount of damage as crabs exposed for 45 min and the depression in metabolic rate was mainly due to Q_{10} effects on metabolism.

All crabs that survived the primary emersion treatments responded to a subsequent period of stress by increasing their oxygen consumption. However, the magnitude of the response following the secondary stressor was significantly lower for animals previously emersed at -15°C than those emersed at 8°C . After the secondary stressor, VO_2 increased from SMR but not over 20% for crabs previously emersed at -15°C , compared to more than 42% for crabs previously emersed at 8°C (Figure 3.2). This indicates that after exposure to -15°C animals were less capable of responding to a

subsequent stressor and were likely compromised. This is especially true for crabs previously emersed for 30 min at -15°C , as they increased VO_2 from SMR by -3%, which was significantly lower than all other treatment groups.

Recovery is generally referred to as the amount of time it takes respiration rates and metabolite levels to return to pre-stress conditions following a stressor and is often used as an indicator of health status (Crear and Forteach, 2001b). In this study, it would appear that all surviving Tanner crabs recovered from the first stressor since VO_2 returned to baseline within 12 hours. However, following a uniform secondary stressor, crabs previously emersed at -15°C could not elevate their respiration rates with the same intensity if at all as compared to crabs previously exposed to 8°C . It is clear that crabs exposed to the more severe treatments were still compromised when considering their metabolic responses to a uniform secondary stressor. Other studies have shown that the effects of cumulative stressors on physiological and behavioral responses of crustaceans and fish may be additive (Winkler, 1987; Barton, 1997). Results illustrate that considering metabolic responses to a single stressor is not always the best index of the overall health and future performance of crabs. However, considering metabolic responses and the magnitude of the response to cumulative stressors might be a better approach.

In conclusion, these data illustrate changes in rates of oxygen consumption following exposure to handling, emersion and severe subfreezing temperature in a high latitude crustacean. We found that the duration and intensity of the stressor had a strong impact on performance of Tanner crabs. While exposure to severe subfreezing temperature was associated with lower survival and depressed VO_2 , milder temperature and durations resulted in increased VO_2 and 100% survival. Thus, differences in rates of oxygen consumption corresponded with differences in survival between the two temperature treatments. The physical stressors are modeled after the type of events that

occur in the commercial crab fisheries. Although crabs may survive capture, they are likely compromised and vulnerable to further periods of stress. Decreased viability of crabs returned to sea may have an impact on future recruitments. Therefore, any changes in the means of harvest that could increase survivorship of bycatch would benefit sustainable fisheries.

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Table 3.1 Percentage survival (S) of Tanner crabs for all sampling periods.

Crabs were exposed to subsequent emersion treatments and mortality was determined at each sampling period: SMR (standard metabolic rate), SPI (immediately after 1°stressor), SPII (12 h after 1°stressor), SPIII (immediately after 2°stressor), and SPIV (12 h after 2°stressor). Distinct letters show significant differences within each column at $P < 0.05$.

T (°C)	D (min)	SMR		SPI		SPII		SPIII		SPIV	
		n	% S	n	% S	n	% S	n	% S	n	% S
8	15	8	100.0	8	100.0	8	100.0	8	100.0 ^a	8	100.0 ^a
8	30	10	100.0	10	100.0	10	100.0	10	100.0 ^a	10	100.0 ^a
8	45	8	100.0	8	100.0	8	100.0	8	100.0 ^a	8	100.0 ^a
-15	15	8	100.0	8	100.0	8	100.0	8	100.0 ^a	8	100.0 ^a
-15	30	10	100.0	10	100.0	10	100.0	10	100.0 ^a	10	100.0 ^a
-15	45	8	100.0	8	100.0	7	87.5	3	37.5 ^b	2	25.0 ^b

T temperature of emersion (°C); D duration of emersion (min); n sample size

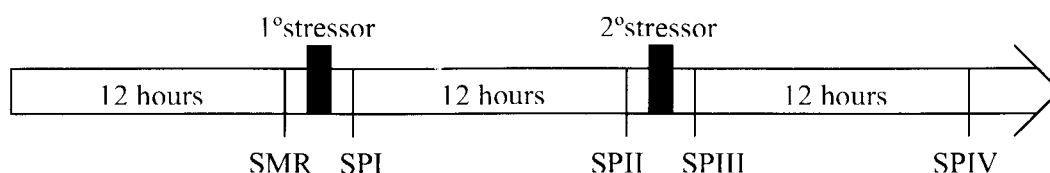


Figure 3.1 Timeline for measurement of rates of oxygen consumption before and after Tanner crabs were exposed to subsequent emersion treatments.

The primary stressor consisted of 1 of 6 treatments: emersion for 15, 30 or 45 min at 8 or -15°C. The secondary stressor was similar for all crabs: emersion for 10 min at -15°C. VO_2 of crabs was determined at each sampling period: SMR (standard metabolic rate), SPI (immediately after 1°stressor), SPII (12 h after 1°stressor), SPIII (immediately after 2°stressor), and SPIV (12 h after 2°stressor).

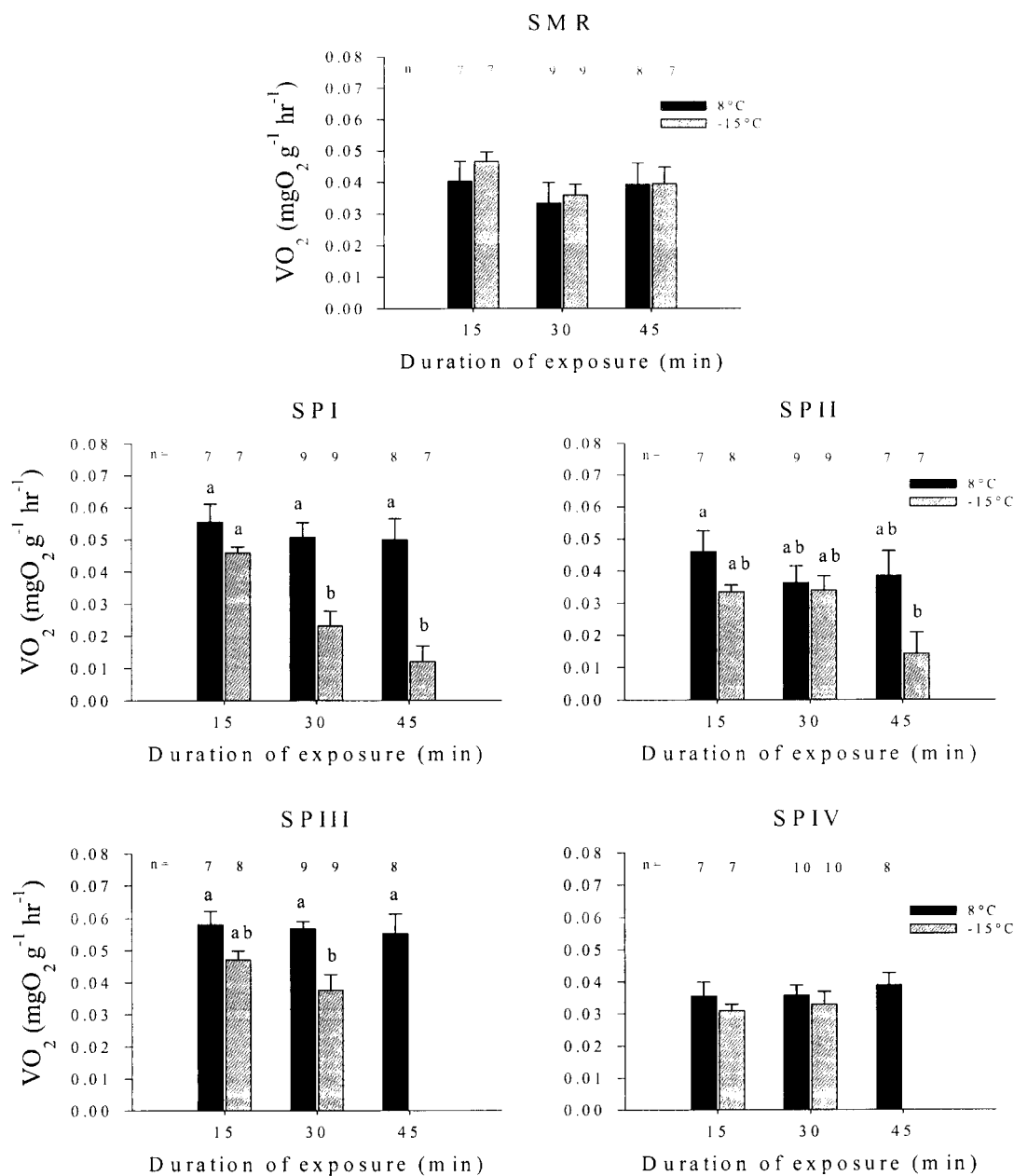


Figure 3.2 Rates of oxygen consumption of Tanner crabs at different sampling periods.

Crabs were exposed to subsequent emersion treatments and VO_2 was determined at each sampling period: SMR (standard metabolic rate), SPI (immediately after 1st stressor), SPII (12 h after 1st stressor), SPIII (immediately after 2nd stressor), SPIV (12 h after 2nd stressor). Vertical bars represent mean VO_2 (+ SE). Means with different letters show significant differences between treatment groups at $P < 0.05$.

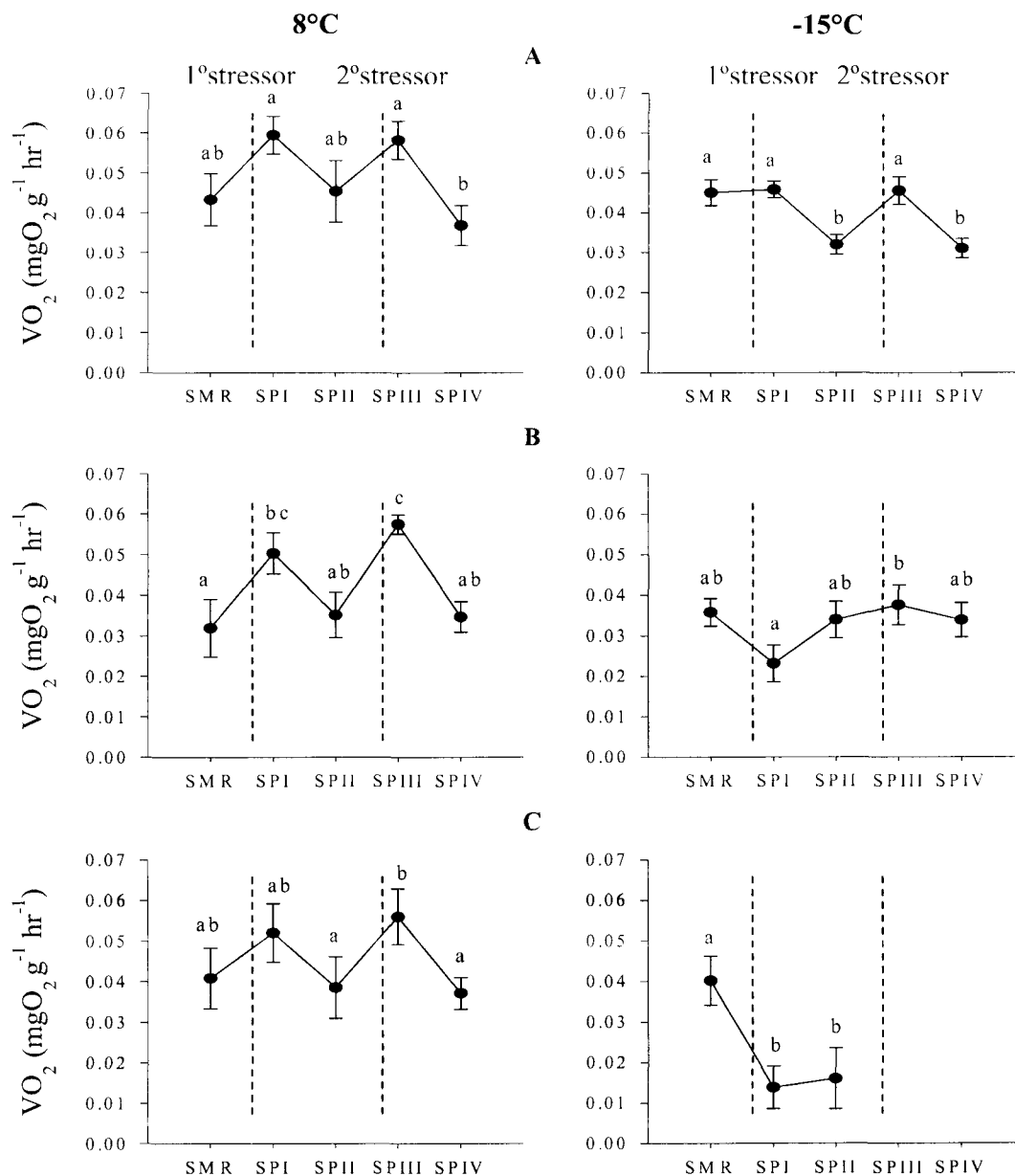


Figure 3.3 Effects of subsequent stressors on rates of oxygen consumption of Tanner crabs previously emersed at 8°C and -15°C for (A) 15 min, (B) 30 min, and (C) 45 min.

X-axis represents the sampling periods: SMR (standard metabolic rate), SPI (immediately after 1^ostressor), SPII (12 h after 1^ostressor), SPIII (immediately after 2^ostressor), SPIV (12 h after 2^ostressor). Scatter plots represent mean VO₂ (\pm SE; $n = 6-9$). Means with different letters show significant differences between sampling periods at $P < 0.05$.

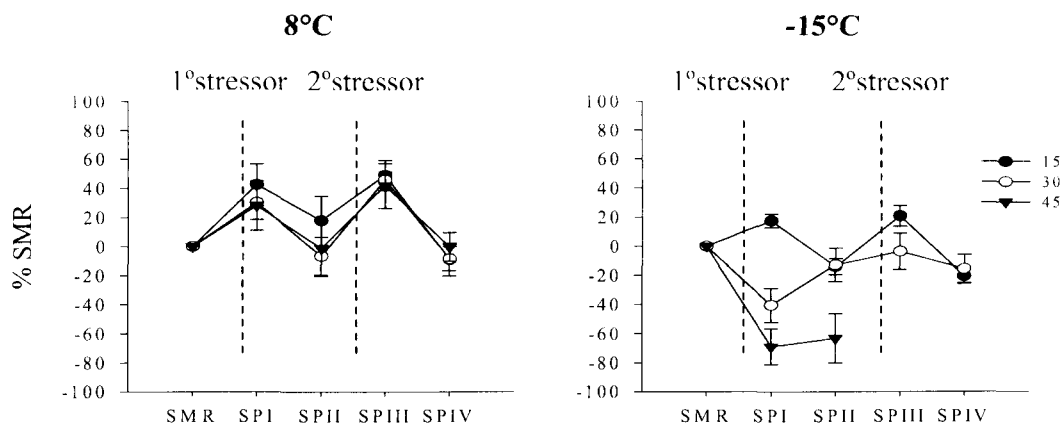


Figure 3.4 Percentage change in rates of oxygen consumption of Tanner crabs.

Scatter plots represent the change in VO_2 (\pm SE; $n = 7-10$) of crabs that were exposed to different durations of emersion (min) at 8°C and -15°C. Change in VO_2 was calculated from the average SMR of undisturbed crabs and is expressed as % SMR. X-axis represents the sampling periods: SMR (standard metabolic rate), SPI (immediately after 1ststressor), SPII (12 h after 1ststressor), SPIII (immediately after 2ndstressor), SPIV (12 h after 2ndstressor).

CHAPTER 4 : GENERAL CONCLUSION

During the course of this study we wanted to evaluate survivorship and performance of Tanner crabs exposed to acute physical stressors associated with commercial crab fisheries while monitoring metabolic indices. Physical insults include exposure to air, subfreezing temperatures, and handling for different durations. By characterizing responses to stress and the time to recover, one can better understand the consequences of the physical insults imposed upon crabs captured. While the sum of these stressors may lead to diminished performance and vitality of Tanner crabs, little data describing their physiological responses to various stressors is available.

To define the range of metabolic responses of Tanner crabs following exposure to mild (emersion at 8°C) and severe (emersion at -15°C) treatments, we measured rates of oxygen consumption and concentrations of glucose and lactate in hemolymph. Our goal was to evaluate the severity of the stressors through metabolic responses, and assess whether these variables could predict mortality.

Results indicate that:

- The duration and intensity of the stressor has a strong impact on the performance of Tanner crabs as seen with low survival, low righting response and increased limb loss
- Exposure to a severe stressor is associated with decreased rates of oxygen consumption, lower hemolymph glucose and increased hemolymph lactate concentrations
- Exposure to a mild stressor results in increased rates of oxygen consumption and 100% survival

- **Thus, physiological differences corresponded with differences in survival between the two temperature treatments**

These data are important for the development of methods that will reduce the stressors on Tanner crabs during capture and handling in current fishing practices. Crabs may survive capture but they are likely compromised and vulnerable to further periods of stress. Any decrease in viability of bycatch crabs may have an impact on future recruitments. Thus, changes in means of harvest that could increase survivorship of bycatch would benefit sustainable fisheries. Moreover, understanding the physiological tolerance to single or cumulative stressors is an important part of the data-base of species-habitat relationships. This information can be used to evaluate effective fisheries resource management, aquaculture practices, and possibly live-shipment of crabs to markets.

APPENDICES

Supplementary data for Chapters 2 and 3

Appendix A

Average daily seawater temperatures ($^{\circ}\text{C}$) in crab holding tanks at the Kodiak Fisheries Research Center in Kodiak, Alaska, in 2002 and 2003

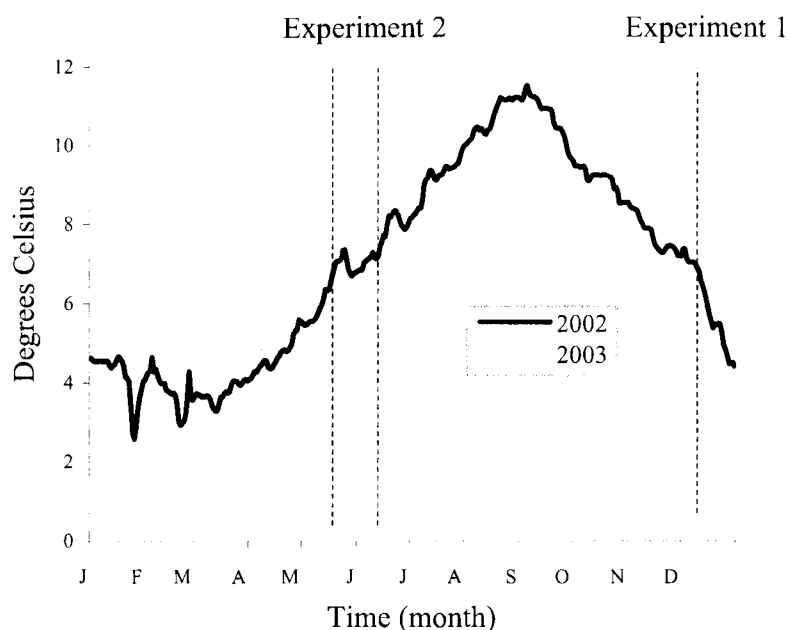


Figure A.1 Average daily seawater temperatures in crab holding tanks in 2002 and 2003.

Holding tanks were supplied with 100% sand-filtered seawater drawn from 25 meters beneath the surface of Trident Basin, Kodiak Alaska, at ambient outdoor water temperature. In December 2002, we collected hemolymph of Tanner crabs following treatments (Experiment 1) for the determination of concentrations of glucose and lactate. Between May and June 2003, we measured rates of oxygen consumption of Tanner crabs following treatments (Experiment 2).

Appendix B

Summary of ANOVA tables for concentrations of glucose and lactate in hemolymph of Tanner crabs

Table B.1 Two-way ANOVA (F-test) for levels of hemolymph glucose between crabs emerged at 8°C or -15°C and sampled at various times post-treatment, on Day 0.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Temperature of emersion (°C)	1	273.491	9.7652	0.003506
Time sampled (min)	2	52.793	1.8850	0.166512
Temperature*Time	2	6.683	0.2386	0.788947
Error	36	28.007		

Table B.2 Two-way ANOVA (F-test) for levels of hemolymph lactate between crabs emerged at 8°C or -15°C and sampled at various times post-treatment, on Day 0.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Temperature of emersion (°C)	1	0.621	0.0261	0.872576
Time sampled (min)	2	11.765	0.4939	0.614297
Temperature*Time	2	101.852	4.2762	0.021566
Error	36	23.818		

Table B.3 T-test for glucose and lactate levels in hemolymph between crabs handled and undisturbed, on Day 0.

	<i>Effect</i>	<i>Degrees of freedom</i>	<i>T</i>	<i>P</i>
Hemolymph Glucose	Treatment	1	-2.64146	0.022933
	Error	11		
Hemolymph Lactate	Treatment	1	-0.377019	0.712742
	Error	12		

Table B.4 Kruskal-Wallis ANOVA on ranks (*H*-test) for levels of glucose and lactate in hemolymph between crabs emerged at 8°C and -15°C, handled and undisturbed, 7 days post-treatment.

<i>Treatment groups</i>	<i>Hemolymph Glucose</i>			<i>Hemolymph Lactate</i>		
	<i>Code</i>	<i>N</i>	<i>Sum of Ranks</i>	<i>Code</i>	<i>N</i>	<i>Sum of Ranks</i>
Emersion at 8°C	103	19	405.0000	103	19	517.5000
Emersion at -15°C	104	15	398.5000	104	14	304.0000
handled	105	7	130.5000	105	7	155.0000
undisturbed	111	7	242.0000	111	7	151.5000

Appendix C

Impact of the combined effects of subfreezing temperature and duration on leg and core temperatures of Tanner crabs

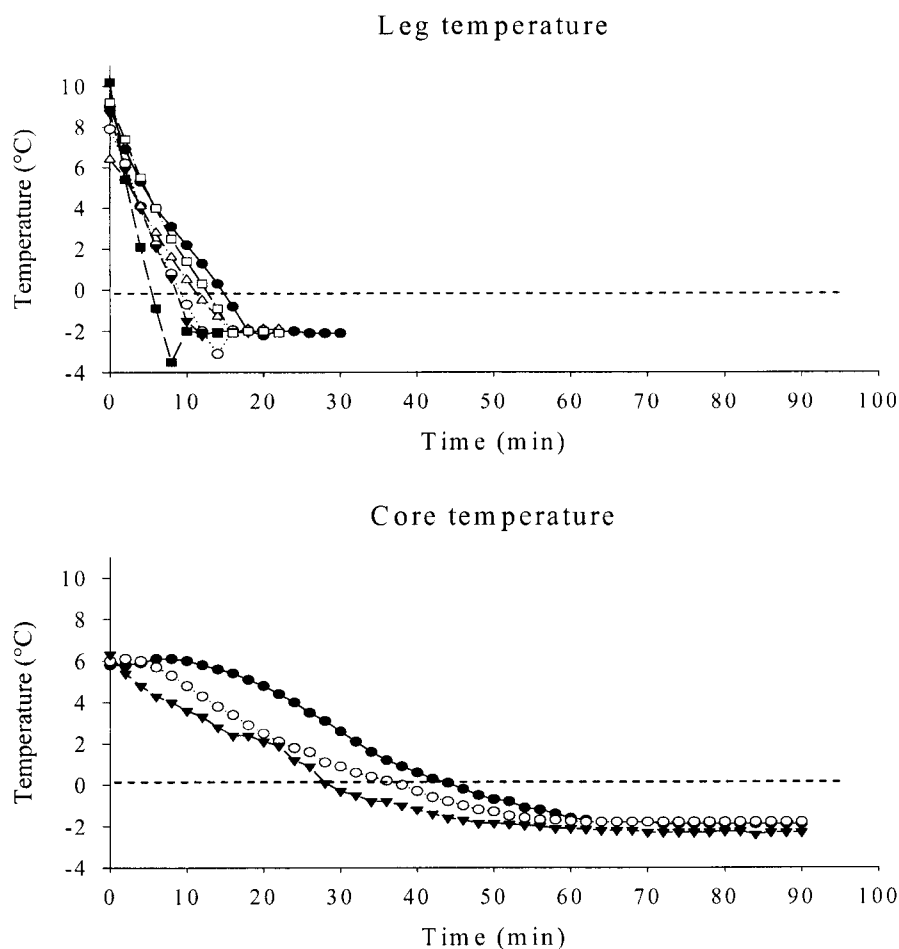


Figure C.1 Leg and core temperatures of Tanner crabs exposed to air at -15°C .

A temperature probe was inserted inside the merus of a leg ($n = 6$ crabs; weights 0.28-0.74 kg), or through the epimeral line at the rear margin of the carapace of a crab ($n = 3$ crabs; weights 0.46-0.94 kg). Crabs were exposed to air at $-15 \pm 0.7^{\circ}\text{C}$ for up to 100 min and leg and core temperatures were measured at 2 min intervals.

Appendix D
Linear drift of the oxygen probes

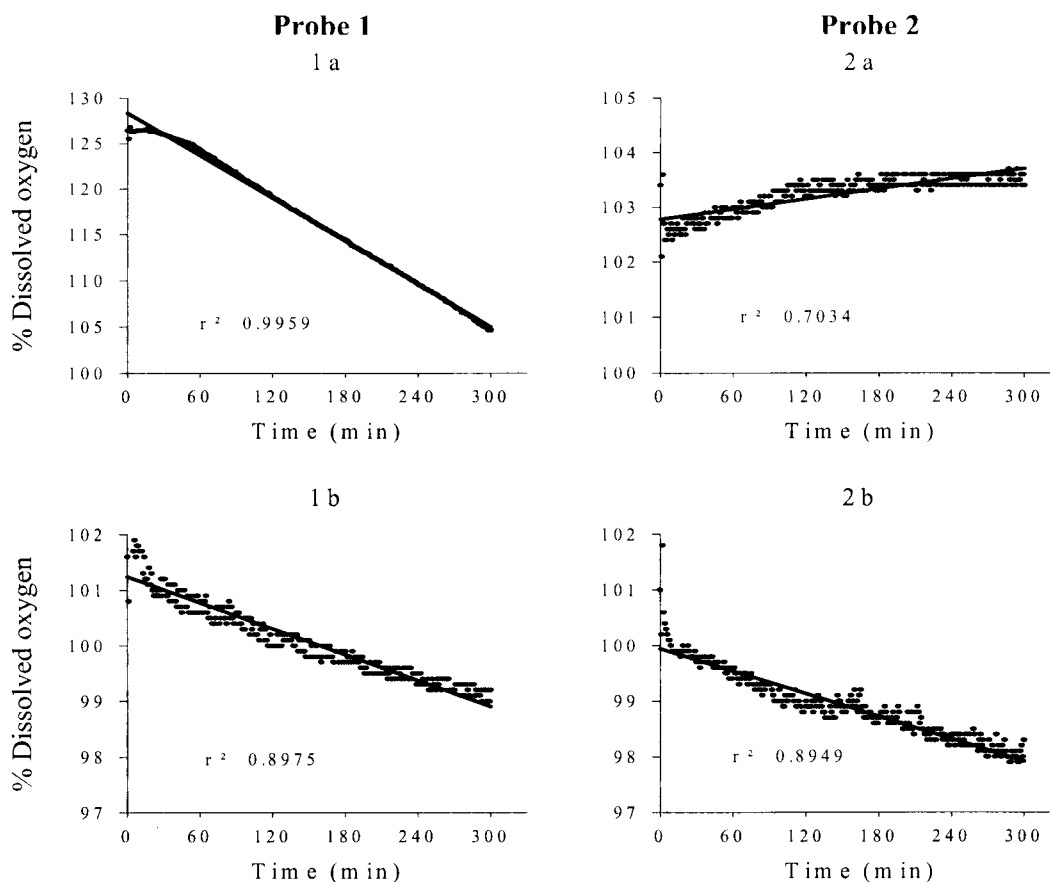


Figure D.1 Percent dissolved oxygen measured for 5 hours in aerated-seawater.

Oxygen probes 1 and 2 were placed in oxygen-saturated seawater and the percent dissolved oxygen (% DO) was measured for 5 hours. This was repeated 5 times for each probe (a, b, c, d, and e). A change in % DO that exceeds $\pm 2\%$ indicates that there is a drift in the oxygen probe. The regression line and $r^2 > 0.85$ show that drifts are linear.

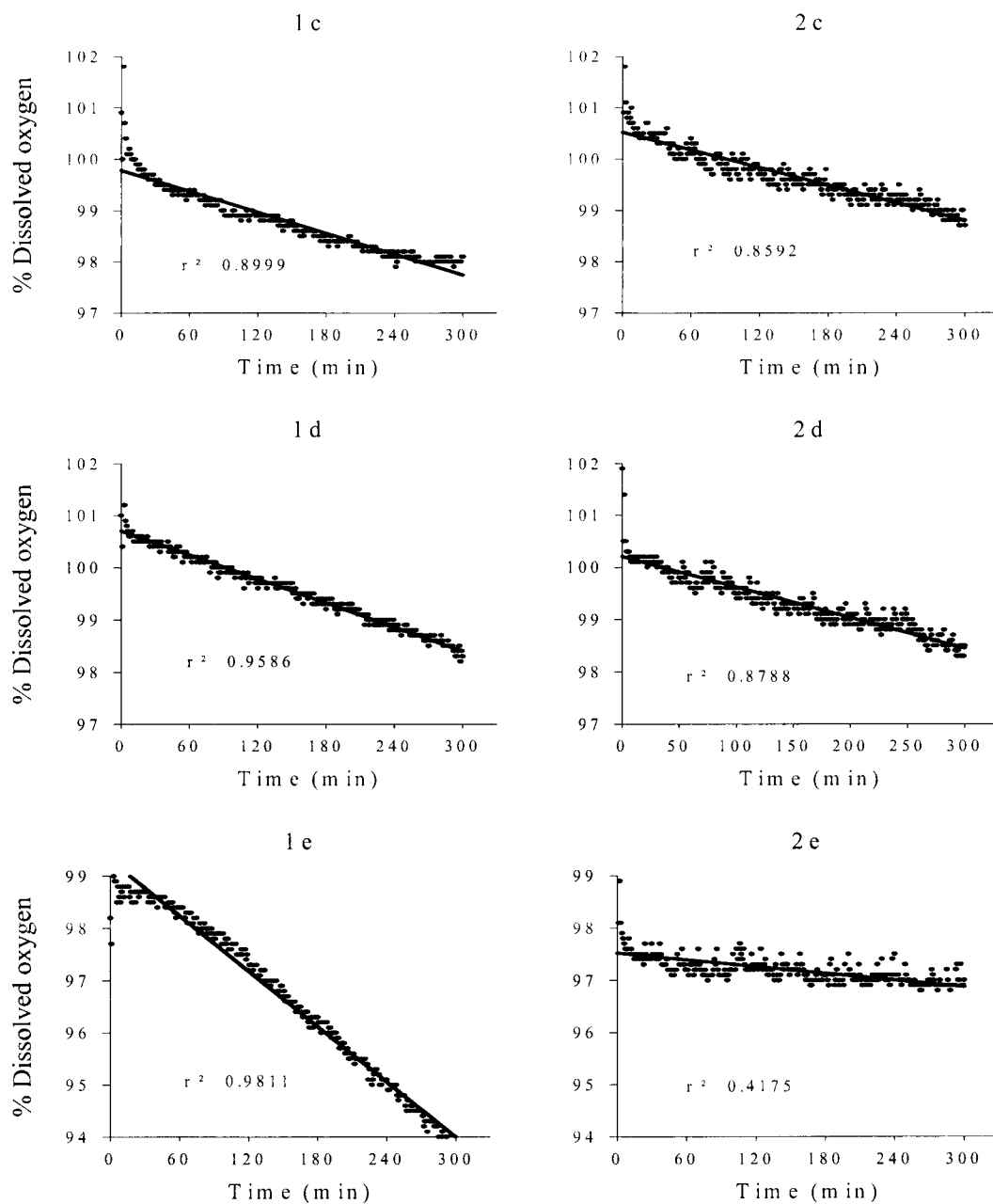


Figure D.1 continued.

Appendix E

Summary of ANOVA tables for rates of oxygen consumption (VO_2) of Tanner crabs

Table E.1 Standard metabolic rates (SMR) of Tanner crabs in the emersion treatments.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Temperature of emersion ($^{\circ}C$)	1	0.000103	0.4420	0.509861
Duration of emersion (min)	2	0.000311	1.3412	0.272755
Temperature*Duration	2	0.000032	0.1361	0.873113
Error	41	0.000232		

Table E.2 VO_2 in SPI between crabs emersed at $8^{\circ}C$ or $-15^{\circ}C$ for various durations.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Temperature of emersion ($^{\circ}C$)	1	0.007254	38.0664	0.000000
Duration of emersion (min)	2	0.001469	7.7095	0.001438
Temperature*Duration	2	0.000729	3.8256	0.029968
Error	41	0.000191		

Table E.3 VO_2 in SPII between crabs emersed at $8^{\circ}C$ or $-15^{\circ}C$ for various durations.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Temperature of emersion ($^{\circ}C$)	1	0.001969	8.3642	0.006099
Duration of emersion (min)	2	0.000659	2.7986	0.072562
Temperature*Duration	2	0.000470	1.9974	0.148676
Error	41	0.000235		

Table E.4 VO_2 in SPIII between crabs emersed at $8^{\circ}C$ or $-15^{\circ}C$ for various durations.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Emersion treatment	4	0.000634	4.3924	0.005395
Error	36	0.000144		

Table E.5 VO_2 in SPIV between crabs emersed at $8^{\circ}C$ or $-15^{\circ}C$ for various durations.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Emersion treatment	4	0.000070	0.6314	0.643236
Error	37	0.000111		

Table E.6 RM ANOVA for VO_2 of crabs previously emersed at 8°C for 15 min and subjected to a subsequent stressor.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Sampling Periods	4	0.000582	5.1779	0.004984
Error	20	0.000112		

Table E.7 RM ANOVA for VO_2 of crabs previously emersed at 8°C for 30 min and subjected to a subsequent stressor.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Sampling Periods	4	0.001019	7.1236	0.000435
Error	28	0.000143		

Table E.8 RM ANOVA for VO_2 of crabs previously emersed at 8°C for 45 min and subjected to a subsequent stressor.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Sampling Periods	4	0.000509	4.66224	0.006300
Error	24	0.000109		

Table E.9 RM ANOVA for VO_2 of crabs previously emersed at 8°C for all durations of exposure and subjected to a subsequent stressor.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Sampling Periods	4	0.001996	17.1151	0.000000
Error	80	0.000117		

Table E.10 RM ANOVA for VO_2 of crabs previously emersed at -15°C for 15 min and subjected to a subsequent stressor.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Sampling Periods	4	0.000350	7.398	0.000793
Error	20	0.000047		

Table E.11 Friedman RM ANOVA on ranks for VO_2 of crabs previously emersed at -15°C for 30 min and subjected to a subsequent stressor.

<i>Sampling Periods</i>	<i>Average Rank</i>	<i>Sum of Ranks</i>	<i>Mean</i>	<i>Standard Deviation</i>
SMR	2.888889	26.00000	0.035782	0.010232
SPI	1.444444	13.00000	0.023168	0.013695
SPII	3.333333	30.00000	0.033994	0.013487
SPIII	4.333333	39.00000	0.037610	0.014680
SPIV	3.000000	27.00000	0.033944	0.012655

Table E.12 RM ANOVA for VO_2 of crabs previously emersed at -15°C for 45 min and subjected to a subsequent stressor.

<i>Effect</i>	<i>Degrees of freedom</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Sampling Periods	2	0.001281	7.80362	0.009082
Error	10	0.000164		